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NAME OF AUTHOR/*NOM DE L'AUTEUR* TOMMY LEE FREDERICKSEN

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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* DR. R. F. Ruth

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THE UNIVERSITY OF ALBERTA

CHICKEN LYMPHOCYTE ANTIGENS

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled .... Chicken Lymphocyte Antigens ....

submitted by Tommy Lee Fredericksen ....

in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Royal F. Ruth
Supervisor

B.M. Longenecker

Sara E. Zalik

W. C. Mackay

External Examiner

Date September 27, 1976

ABSTRACT

Ring-necked pheasants were immunized with lymphocytes from the thymus and bursa of Fabricius of $\underline{B}^2/\underline{B}^2$ chickens. Pheasant anti-chicken thymus serum (PATS) promoted in vitro adherence of thymus cells and pheasant anti-chicken bursa serum (PABS) promoted the adherence of bursa cells. These reactions were observed for other lymphoid populations in the blood and spleen but to a lesser extent. Each antiserum was able to discriminate between a thymus-related antigen and a bursa-related antigen. This reaction was complete after 60 minutes of incubation with PATS and after 120 minutes of incubation with PABS. The rate of the reaction was not altered by a slight change in antiserum concentration or changes in temperature of incubation (22° C to 38° C). Incubation of peripheral blood lymphocytes (PBLs) with PATS reduced the level of the graft-versus-host (GvH) immunocompetence of these cells in the chorioallantoic pock assay, a thymus cell function. This result however was not easily reproduced. Incubation of PBLs with PABS did not alter the GvH immunocompetence. PBLs incubated with either PATS or PABS reduced their ability to evoke the GvH splenomegaly reaction. The GvH reducing effect was absorbed with cells derived from the thymus and bursa but not with erythrocytes. The ability of these antisera to inhibit the GvH reactivity of lymphocytes has been utilized as a method for the identification of a chicken lymphocyte antigen marker.

This assay system has provided the framework for defining a new T-cell lymphocyte alloantigen system.

Two inbred lines of chickens (L6 and L7) homozygous at the *B* locus were immunized with each others blood cells. Anti-L6 antiserum (A6) prepared in L7 recipients limited the GvH splenomegaly evoked by L6 PBLs and anti-L7 antiserum (A7) prepared in L6 recipients limited the GvH spleen weight evoked by L7 PBLs. Both antisera were able to limit the GvH splenomegaly evoked by F1 PBLs. These results can be explained by postulating the existence of a new lymphocyte antigen locus. As a test of this possibility L6 individuals were assumed to be homozygous for one allele and L7 homozygous for the other. Predetermined limits were set to distinguish these antigen types in 101 F2's. The PBLs of 22 F2's were classified as L6-type, 52 as F1-type, and 27 as L7-type. A probability graph confirmed the division of the F2's into 3 populations. Since the limiting effect of these antisera was not absorbed by erythrocytes or lymphocytes derived from the bursa, the antigen appears to be distinct from the A, B, C, and Bu-1 systems. Tests with selected F2 chickens indicated that this antigen system is not identical to Th-1 or the B antigen systems. This antigen system is therefore designated Ly-4 and its alleles are *Ly-4*^{a} for L6 and *Ly-4*^{b} for L7. Preliminary studies in tests of F3 progeny indicate a possible association between donors of the L6-type and resistance to MD, and of donors of the L7-type and susceptibility to MD.

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Chapter 1

Detection of Lymphocyte Antigens with Pheasant Antiserum

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INTRODUCTION

Lymphocytes have been characterized according to morphological, physical, chemical, antigenic, and functional criteria. Each of these criteria attest to the basic fact that lymphocytes are a heterogeneous class of cells comprising the essential components of the immune system. One of these criteria, the functional capacity of lymphocytes in the generation of immunological responsiveness provides a reference point for judging the scientific value of the other four criteria. In this context then, the work presented in this thesis focuses upon establishing new antigenic criteria which may be used to identify different classes of lymphocytes in the chicken.

The immune system of the chicken as well as that of mammals is comprised of two functionally distinct lymphoid cell populations. In the late 1950's, and early 1960's, the importance of the role of chicken thymus-derived lymphocytes (Th cells) in the development of cell-mediated immunity (defined by skin homograft and graft-versus-host reactions) and of chicken bursa-derived lymphocytes (Bu cells) in the development of antibody-mediated immunity (defined by antibody production after inoculation with bacterial and protein antigens) was established using the techniques of neonatal thymectomy or bursectomy to eliminate the source of lymphoid cells arising from these organs (Aspinall and Meyer 1964; Cooper *et al.*, 1966; Glick 1964; Mueller *et al.*, 1964; Warner and Szenberg 1964). In the mouse, the functional dissociation of immunological responsiveness for cell-mediated immunity was established using the technique of neonatal thymectomy to selectively deplete the immune system of thymus-derived lymphocytes (T cells) (Dalmasso *et al.*, 1964; Miller 1964) and for antibody-mediated immunity using the adoptive transfer system to define the importance of bone marrow-derived lymphocytes (B cells) in the generation of antibody forming cells (Claman *et al.*, 1966; Miller and Mitchell 1968). Birds are different from mammals in the sense that the functional development of immunological responsiveness is associated with the development of lymphocytes in two separate lymphoepithelial organs, the thymus and bursa of Fabricius.

In more recent years, the identification of antigenic cell markers of lymphoid cells which represent products of gene expression has been one of the primary tools used to establish the cellular and functional heterogeneity of T or B lymphoid cell populations. Thus far, the most well characterized system of antigenic cell markers for lymphocytes has been established in the mouse. In mice a total of eleven lymphocyte antigen cell markers have been described excluding those mapping within the major histocompaibility complex (H-2). These are: 1) the thymus leukemic antigen (TL), 2) the theta antigen (Thy-1), 3 through 7) the lymphocyte specific antigens (Ly-1, 2, 3, 4, 5, 8) the mouse specific lymphocyte antigen (MSLA), 9) the plasma cell antigen (PC), 10) the mouse specific B cell antigen (MBLA), and 11) the immunoglobulin determinant antigen (ID). All eleven antigens were detected on lymphocytes using the complement-dependent cytotoxicity assay. The antigen markers defined with alloantisera (antisera raised against cells from within the same species) are strain specific (TL, Thy-1, Ly, PC) while those defined with heteroantisera (antisera raised against cells from other animal species) are species specific.

The TL antigen is expressed on lymphocytes of the thymus of TL^+ mice strains and leukemic cells residing outside the thymus of both TL^+ and TL^- mice (Old) U et al., 1963; Boyse *et al.*, 1965; 1969). The significance of this marker resides in the fact that in TL^+ strains, T cells of the peripheral lymphoid system do not express the TL antigen and it has been suggested therefore that either the 5% TL^- lymphocytes in the thymus give rise to peripheral T cells, or that the TL^+ lymphocytes undergo a process of differentiation and in that process become TL^- prior to entering the peripheral lymphoid system (reviewed in Schlesinger 1972). Anti-TL serum is produced by immunization of TL^- mice with TL^+ thymus cells. The antiserum's activity is measured in the cytotoxicity assay using guinea pig serum as a source of complement. The *Tla* locus appears to perform the function of a regulator gene coding for the expression or absence of expression of the TL phenotypes while another locus or loci (possibly one of the *Ly* loci, Komuro *et al.*, 1975) operates as structural genes coding for the Tl phenotypes. The _TLa_ locus is linked with the _H_-2 locus on chromosome 17.

The theta, Ly-1, 2, 3, and 5 antigens are expressed on thymus and peripheral T cells. Evidence supporting the conclusion that these antigens are markers for T cells come from experiments which demonstrate _in vitro_ the absence or disappearance of these cells from the peripheral lymphoid system after neonatal thymectomy, adult thymectomy and in congenitally athymic, nude mice (Raff 1969; Raff and Wortis 1970; Schlesinger and Yron 1970; Raff 1971). Anti-theta serum is raised by immunizations of AKR mice with C_3H mouse thymus cells and vice versa (Reif and Allen 1963; 1964; Aoki _et al_., 1969; Raff 1971). The antiserum activity is measured in the cytotoxicity assay using guinea pig serum as a source of complement. The theta antigen is also expressed on some non-lymphoid cells of the central nervous system. The specificity of this antiserum is altered in the cytotoxic reaction with lymphocytes if rabbit serum is substituted for guinea pig serum as a source of complement. In this situation the antisera will define antigen markers common to both T and B lymphocytes (Greaves and Raff 1970). The _Thy_-1 locus, controlling the expression of the theta antigen, has been mapped on chromosome 9 (Itakura _et al_., 1971) and the two alleles associated with this locus account for the only known types of theta antigen markers identified in laboratory strains of mice.

The Ly-1, 2, and 3 antigen markers are believed to identify subpopulations of theta-bearing lymphocytes in the peripheral lymphoid organs (Cantor and Boyse 1975a). In addition to serological evidence which supports this conclusion, the use of these sera to selectively kill Ly-1 or Ly-2, 3 antigen bearing peripheral T cells (Cantor and Boyse 1975a; 1975b) has resulted in the findings that lymphocytes expressing the Ly-1 alloantigen marker appear to be involved in the generation of helper cell activity, the amplification of killer cell activity directed against major histocompatibility differences, and in the proliferative response measured in the mixed lymphocyte culture (MLC) response when there is an *I* region incompatibility. On the other hand, those lymphocytes expressing Ly-2, 3 alloantigen markers appear to play a role in the generation of killer cells and in the proliferative response measured in the MLC response against H-2K and H-2D incompatibility. Anti-Ly-1.1 and anti-Ly-2.2 sera are prepared in congenic lines of mice differing at the *Ly* locus using thymus cells (Shen *et al.*, 1975). Anti-Ly-1.2, 2.1, 3.1 and 3.2 sera are prepared in noncongenic mice using thymus cells (Boyse *et al.*, 1968; 1971; Shen *et al.*, 1975). The antiserum's activity is measured in the cytotoxicity assay using rabbit serum as a source of complement. The *Ly*-1 locus has been mapped on chromosome 19 and the *Ly*-2, 3 loci are tightly linked on chromosome 6 (Itakura *et al.*, 1971; 1972).

The Ly-5 antigen marker was recently identied by Komuro *et al.*, 1975. Anti-Ly-5 serum is prepared between mouse strains compatible for H-2 antigens by immunizing with lymph node or spleen cells. Two alleles have been identified at the _Ly_-5 locus which segregates independently from the other lymphocyte alloantigen loci described above. Finally, the seventh antigen marker (MSLA) identified on T cells of the thymus, peripheral T cells, and on some leukemic cells was described by Shigeno _et al_., 1968. Anti-MSLA serum is prepared by immunizations of rabbits, guinea pigs, or rats with mouse thymus cells. The antiserum's activity is defined in the cytotoxicity assay using guinea pig serum as a source of complement. The MSLA marker for T cells is species specific and therefore found on T cells of all mouse strains.

B cell markers in the mouse are identified in terms of: 1) the antithetical relationship that exists in the distribution of T and B antigens on a lymphocyte, ie. the expression of T or B antigen on a lymphocyte appears to be mutually exclusive; 2) the retention or quantitative increase in the number of lymphocytes that express the B cell antigen marker in neonatally thymectomized mice or athymic, nude mice; and 3) the ability of anti-B serum to suppress B cell mediated immune responsiveness. The Ly-4, MBLA, and ID antigen markers are represented on B cells, B-derived cells, antibody producing cells, and on tumor cells of the plasma cell type. Anti-Ly-4 serum is produced by immunization with lymph node or spleen cells between mice strains which are Ly-4.2^{+} and Ly-4.2^{-} (Snell _et al_., 1973;

Aoki *et al.*, 1974; McKenzie 1975; McKenzie and Snell 1975). The cytotoxic activity of anti-Ly-4 serum is obtained in the presence of rabbit complement. Thus far, the Ly-4 antigen marker is the only alloantigenic B cell system that has been described. The MBLA antigen is identified with heteroantisera raised in rabbits and produced against lymph node cells obtained from thymectomized mice (Raff 1971). The cytotoxic reactivity of this antiserum requires the addition of guinea pig complement. Anti-ID serum is produced by immunizing rabbits with mouse kappa light chain immunoglobulin, with mouse immunoglobulin obtained from serum or from experimentally induced myelomas, plasma cell tumors (Raff *et al.*, 1970; Takahashi *et al.*, 1971). The ID cell markers appear to represent a universal cell marker for B-cells in all avian and mammalian systems. Finally, the PC antigen marker represents a unique B cell antigen marker because it is only expressed on antibody producing cells and plasma cells of myelomas (Takahashi *et al.*, 1970; 1971). Anti-PC serum is raised in rabbits using purified myeloma or urinary protein and the cytotoxic activity of the serum is obtained in the presence of guinea pig complement.

Antigenic analysis of cells in the lymphoid tissue have revealed the following T and B cell organ distributions in the mouse: 1) The thymus is comprised of 95 to 100% T cells and 0 to 5% B cells; 2) the bone marrow is comprised of 0% T cells and 39% B cells; 3) the thoracic duct lymphoid population is comprised of 80 to 90% T cells and 14% B cells; 4) the peripheral blood population is comprised of 71% T cell and 33% B cells; 5) the lymph node population is comprised of 60 to 85% T cells and 28% B cells; 6) the spleen is comprised of 25 to 40% T cells and 56% B cells; 7) the peritoneal exudate lymphocyte population is comprised of 25 to 50% T cells and 35% B cells; and 8) the lymphocytes of Peyer's patches are comprised of 20 to 40% T cells and 68% B cells (Raff 1971; McKenzie 1975).

Until very recently only heteroantisera have been used to define Th and Bu cell antigen markers in the chicken. The reasons for this are that the existence and availability of inbred chicken lines are quite limited mainly due to severely reduced fertility and viability of these chicken lines (inbreeding depression). Th and Bu cell antigen markers have been identified on chicken lymphocytes in the complement-dependent immune adherence assay (Forget et al., 1970) and cytotoxicity assay (McArthur et al., 1971; Potworowski et al., 1971; Ivanyi and Lydyard 1972; Wick et al., 1973). Indirect immunofluorescence has also been used in conjuction with anti-Th and -Bu serum raised in rabbits and turkeys to identify these antigen markers on the surface of lymphoid cells (Potworowski et al., 1972; Hudson and Roitt 1973; Wick et al., 1973). Finally, anti-ID serum raised against chicken immunoglobulins in rabbits and turkeys define a second species specific antigen marker for Bu cells (Bankhurst et al., 1971; Albini and Wick 1973).

Antigen analysis of cells in chicken lymphoid tissue thus far have revealed the following Th and Bu cell distributions: 1) The thymus is comprised of 90% Th cells and 10% Bu cells; 2) the bursa is comprised of 0 to 5% Th cells and 76 to 96% Bu cells; 3) the spleen is comprised of 56 to 58% Th cells and 38 to 41% Bu cells; and 4) the peripheral blood is comprised of 57 to 59% Th cells and 23 to 41% Bu cells (Hudson and Roitt 1973; Wick et al., 1975).

The objective of this study was to identify and define new chicken lymphoid antigen markers. The approach takes into consideration that: 1) during ontogeny Th- and Bu-derived cell populations arise from cells developing in the thymus and bursa of Fabricius, respectively; 2) sensitive assay systems (the chorioallantoic membrane pock assay and splenomegaly assay) already exist for examining the role of lymphoid cells in the mediation of cellular immunity against transplantation antigens; and 3) heteroantisera produced in rabbits against Th or Bu cells do not provide an adequate tool for *in vivo* examination of the Th and Bu cell functions nor for the identification of lymphoid alloantigen systems. The contents of chapter one examine the potential capacity of heteroantisera, raised in pheasants (a distant relative of the chicken) against chicken Th and Bu cells, to be used in the detection of Th and Bu cell antigen markers on chicken lymphoid cells and their possible application toward the identification *in vivo* of the role of Th-derived cells in the generation of a graft-versus-host (GvH) response against major transplantation antigens. During the course of this examination, the ability of the heteroantisera to inhibit the GvH reactivity of lymphocytes has been utilized as a tool for the identification of a chicken lymphocyte antigen marker common to thymus, bursa, and peripheral blood lymphocytes. This assay system has provided the basis for the identification of a new lymphocyte alloantigen system described in chapter two.

MATERIALS AND METHODS

I. Animals

Ring-necked pheasants were obtained at 6 weeks of age from a local supplier. The pheasants were housed in individual chicken cages and fed chick starter, grit, and water *ad libitum*. $\underline{B}^{2}/\underline{B}^{2}$ and $\underline{B}^{14}/\underline{B}^{14}$ genotype White Leghorn chickens were used as donors in the GvH reaction. The genetic stock was originally acquired from Hyline International, Johnston, Iowa. WC ($\underline{B}^{2}/\underline{B}^{2}$) eggs obtained from Hyline International and outbred eggs obtained from the University of Alberta poultry farm, Edmonton, Alberta, were used as recipients in the GvH chorioallantoic membrane (CAM) pock assay and splenomegaly assay.

II. Preparation of Cells

A) Thymocytes: Six to 10 thymus lobes from 2-4 week-old chicks were carefully excised from the right and left carotids so as not to contaminate the tissue with erythrocytes. The tissue was placed in a test tube containing Dulbecco's phosphate buffered saline (pH=7.4) and kept at 4° C. Cell suspensions were prepared by gently teasing pieces of the thymus tissue, using a small sterile curved forceps, through a small wire screen immersed in Eagles minimal essential medium (MEM) with Hank's balanced salts supplemented with 1% normal or agamma chick serum (NS) (North American Biological, Inc.), 20 mM of Hepes buffer, 50 ug/ml of penicillin-streptomycin, and 50 ug/ml of mycostatin. This cell suspension was then passed through a 20 gauge needle and placed in 17x100 mm sterile plastic tubes. Large clumps of cells were removed by a settling out process during a five minute incubation period at room temperature. The supernatant was discarded and the cells were washed two more times using the MEM's described above. The washed cells were stored at 4° C.

B) Bursa: The bursa of Fabricius was excised carefully from 2-4 week-old chicks. Bursa cell suspensions were prepared in the manner described for thymus cells.

C) Spleen cells: The spleen was removed aseptically from $\underline{B}^2/\underline{B}^2$ donors and pressed through a small wire screen placed in the barrel of the syringe using the syringe plunger. These cells were then prepared in the same manner as described for thymus cells.

D) Peripheral blood lymphocytes (PBLs) and erythrocytes (RBCs): Four to 10 mls of heparinized (10 units/ml) peripheral blood were obtained from appropriate donors and centrifuged individually in 17x100 mm plastic tubes at 600 rpm (66 g) for 10 minutes. The lymphocyte rich plasma, buffy coat, and 2 to 4 mm of the upper RBC layer was collected. Chicken RBCs were collected from the bottom 5 to 10 mm of the same blood sample. The PBLs were then separated from the RBCs using a modification of the Bøyum technique (Bøyum 1968). Two mls of Seligmann's balanced salt solution containing 15 mM Hepes was used to dilute 2 mls of this PBL-RBC suspension. Four mls of the diluted cell suspension was then layered onto 3 mls of lymphoprep (Nyegaard and Co.) or lymphocyte separating medium (Litton Bionetics, Inc.) in 17x100 mm plastic tubes. The cells were then centrifuged at 1500 rpm (413 g) for 20 minutes and the PBLs were collected at the interface between the lymphoprep and Seligmann's solution-chicken plasma mixture. For each 2 mls of the PBL suspension 10 volumes of phosphate buffered saline were added, mixed thoroughly, and centrifuged at 1800 rpm (595 g) for 10 minutes. The supernatant was discarded and MEM with supplements was added. In this preparation as well as in the preparation of thymus and bursa cells, the lymphocyte concentration was enriched by twice incubating these cells in 60x15 mm petri plates at room temperature to remove contaminating granulocytes, thrombocytes, and monocytes (Wong *et al.*, 1972). The PBLs were usually prepared the day prior to embryo inoculation and stored overnight at 4^{0} C. The cell suspensions were counted with a Fisher autocytometer II cell counter or with a cell hemocytometer using a phase contrast microscope.

III . Preparation of Pheasant Antithymus Serum (PATS) and Antibursa Serum (PABS)

A) Antisera: $\underline{B}^{2}/\underline{B}^{2}$ thymus cells ($2-4x10^{8}$ cells) or bursa cells ($1-2x10^{8}$ cells) diluted in phosphate buffered saline were mixed with complete Freund's adjuvant (Microbiological Associates) in a 4:1 ratio of cell suspension to

Freund's. This mixture was injected intraperitoneally (i.p.) into 2-3 month-old pheasants. Immunizations were given at bimonthly intervals for a period of 2 to 3 months. Six days following the last immunization, the pheasants were bled for plasma from the wing vein. After a resting period of 2 to 3 weeks, these pheasants were immunized two to three more times and bled once again. The heparinized blood was centrifuged at 1500 rpm (413 g) to pellet the red and white cells and the plasma fraction was collected and stored in 2 to 3 ml aliquots at a temperature of -90° C.

B) Absorption of the antisera: A 2 to 3 ml aliquot of putative antiserum was thawed and heat-inactivated at 56° C for 30 minutes. Each aliquot of antiserum was then diluted with an equal volume of phosphate buffered saline. Each antiserum was absorbed with $2\text{-}4\times10^8$ $\underline{B}^2/\underline{B}^2$ RBCs for a period of 10 minutes at room temperature. The RBCs were then removed by centrifugation and the procedure was repeated twice more. The absorptions with RBCs (which in the chicken carry histocompatibility antigens) is to remove erythrocyte heteroantibodies and anti-B2 antibodies. PATS were then absorbed twice using 0.8 ml of packed bursal cells. PABS were absorbed twice using 0.8 ml of packed thymus cells. These absorptions were carried out at 22° C for a period of 45 minutes on a rotating shaker (150 rpm). To each 2 ml aliquot of antiserum 100 ug/ml of gentamycin (Microbiological Associates) was added and the antiserum was then stored at -90° C until further use.

IV. Assays for Antilymphocyte Activity

A) Cytotoxicity assay: The cytotoxicity assays were performed in sterile 12x75 mm glass tubes containing 0.1 ml of the appropriate cells (at a concentration of 2×10^6 cells/ml) in MEM with supplements and 0.05 ml of fresh normal chicken serum (NCS) or fresh normal pheasant serum (NPS) as a source of complement. To each tube 0.15 mls of serially diluted (with phosphate buffered saline) antiserum was added and incubated at 37^0 C for 1 hour. After this incubation period, 0.3 ml of 0.4% trypan blue dye solution was added to each tube and the number of unstained, ie. viable, cells was counted in a hematocytometer. The percent cytotoxicity was calculated as: (number of viable cells counted after incubation with antiserum divided by the number of viable cells counted after incubation with NCS or MEM with supplements)x100.

B) Agglutination assay: The agglutination assays were performed in microtiter plates (Cooke Laboratory Products). Serially diluted antiserum (0.2 ml) was added to 0.05 ml of the appropriate lymphocytes (10^7 cells/ml) or RBCs (2% v/v). The plates were then incubated at 37^0 C for a period of 75 minutes and the titers were read microscopically in the lymphocyte agglutination assay and macroscopically for the erythrocyte agglutination assay.

C) Lymphocyte adherence test: The lymphocyte adherence test was first described by Wong *et al.*, 1972, and Wong 1974, as an *in vitro* typing assay used to identify major (*B*) and minor (non-B) histocompatibility antigens on lymphocytes. The test is done by mixing lymphocytes, fresh plasma, and alloantibody in a small plastic tube and counting the number of lymphocytes which remain in suspension. After 15 minutes the lymphocytes began to adhere to the surface of the tube and the reaction is completed in 2 hours. The attachment of lymphocytes (referred to as allofixation) occurs under conditions which indicate that the antiserum detects lymphocyte antigens. The percent lymphocyte adherence (LA) was calculated as: (number of cells lost from the supernatant after incubation with antiserum divided by the initial cell counts at the beginning of the test)x100. This test was adopted as a method for identifying thymus (Th)-and bursa (Bu)-specific antigens on lymphocyte populations using the putative PATS or PABS as typing serum. In each 12x75 mm glass culture tube 100ul of MEM with supplements (pH=8.3), 50 ul of the lymphocyte suspension (cell concentration 24×10^6 cells/ml), 25 ul of NCS, and 25 ul of antiserum were added and duplicate tests were conducted for each group. The LA test was also done in large plastic tubes (17x100 mm) containing a total of 1 ml (5 times the volume used in the small tubes) to facilitate using the treated cells in the PBL, GvH assays. The cellular immunocompetence of PBLs was tested in the CAM pock assay as described in Longenecker et al., 1970, using 12 day-old embryos instead of 11 day-old embryos.

D) Splenomegaly inhibition assay: In 12x75 mm plastic tubes 5-10x10^6 PBLs/ml from appropriate donors were incubated for a period of 30 minutes at 4° C or at 22° C with PATS, PABS, or NS. The GvH reactivity of the PBLs from the different treatment groups was tested in the GvH splenomegaly assay (Simonsen 1962; Ruth et al., 1965; Seto and Albright 1965). The serum-cell mixture from each group was injected intravenously (i.v.) into groups of 10 to 13, 12 day-old chick embryos in a volume of 0.1 ml/embryo. Six days later, the embryonic spleens were removed, weighed to the nearest mg, and the spleen weights were transformed to $\log_{10}$ (Longenecker et al., 1972). The $\log_{10}$ spleen weights in the different treatment groups from each donor were compared using Duncan's new multiple range test (Sokal and Rohlf 1969). Other statistical tests used to analyze the data (regression analysis, analysis of variance) were as described in the texts of Steel and Torrie 1960, and Sokal and Rohlf 1969. The specificity of the antisera was tested following the absorption of 1 ml of the prepared PATS or PABS with 10^8 Th, Bu, or RB cells/ml at room temperature for 60 minutes.

RESULTS

I. Cytotoxic and Agglutinin Activity of PATS and PABS

A 1:2 dilution of PATS or PABS was found to be 100% cytotoxic for both Th and Bu cells and at a 1:4 dilution, the cytotoxicity dropped to 25%. The antisera were not specific for either population of cells. At a 1:8 dilution, the cytotoxic activity was absent but both Th and Bu lymphocytes formed large agglutinates. For each successive dilution of antiserum from titres of 1:4 to 1:32, the clump size decreased. No clumps were seen at a dilution of 1:64. The agglutinin activity exhibited by these antisera was not specific for either population of cells. No difference was detected in the cytotoxic or agglutinin titre in tests using freshly thawed or heat-inactivated (at 56^0 C for 30 minutes) antiserum and between tests using chicken or pheasant serum as a source of complement. The unabsorbed antisera did however contain RBC agglutinin activity up to a $\log_2$ titre of 3 or 4 which was easily removed following absorption with RBCs.

II. The Identification of Specific Thymus and Bursa Cell Antigens

The fact that Th and Bu cells were equally reactive in the cytotoxic and lymphoagglutination tests with either antiserum indicates that the antibody activity is directed against an antigen or antigens shared by Th and Bu cells.

The lymphocyte adherence assay was used to attempt to show a specific Th or Bu reaction by PATS or PABS. The adherence for Th cells was between 50 and 80% at 1 hour of incubation with PATS and 5 to 10% with PABS. The dilution curves are similar and parallel on a $\log_{10}$-$\log_{10}$ scale (Figure 1). The adherence of Th cells incubated with NPS was between 2 to 6%. This is not significantly different from the adherence of these cells incubated with PABS. The specific adherence for Bu cells, on the other hand, was approximately 80% after 2 hours of incubation with PABS. These dilution curves are also similar and parallel (Figure 2). A high background of nonspecific adherence (30%) was consistently found in the Bu cell suspensions in the presence or absence of PATS. This high background could be reduced from 30 to 15% by preincubating these cells for 20 minutes in petri dishes at 22° C. This nonspecific adherence correlated with the number of dead cells, those stained with trypan blue, observed in the Bu cell preparation.

The effects of varying the conditions and reagents for adherence were tested for both Th and Bu cells. When normal pheasant serum was substituted for normal chicken serum as a source of complement the adherence in either the presence or absence of antiserum was increased by 8% for Th and 16% for Bu cells (Table I). The addition of NCS to the incubation medium was essential for measuring the PABS activity but not for PATS activity (Table II).

Adherence begins to occur within 15 minutes after incubation of the lymphocytes as shown for the LA of Th cells incubated with PATS in Figure 3 and for Bu cells incubated with PABS in Figure 4. The time curves of Figures 3 and 4 indicate that the rate of adherence is greatest during the first 15 minutes, 25% for Th cells incubated with PATS and 20% for Bu cells incubated with PABS. After this, the LA takes place at a relatively constant rate of 12 to 16% per hour as indicated from the linearity of the time curves. Regression lines were used to approximate the slope of these lines for the purpose of making comparisons between experiments.

Next Th cells were incubated at either 22° C or 38° C with 1:32 (Figure 5a) or a 1:64 (Figure 5b) dilution of PATS. The rates of adherence at 22° C was parallel to, but less than the rates of adherence for 38° C at both antiserum concentrations tested.

The distribution of Th- and Bu-specific antigens on lymphocytes developing in the thymus and bursa of Fabricius was examined in 0 to 56 day-old chicks (Figure 6). Non-significant fluctuations occur in the specific and non-specific adherence of Th or Bu cells with increasing age of the donor. The average adherence observed for Th cells was 59.1% and for Bu cells, 47%. The average nonspecific adherence for Th cells incubated with PABS of the varying aged donors was 6.5% and for Bu cells incubated with PATS, 17%. The distribution of the Th- and Bu-specific antigens was also examined on lymphocytes obtained from the spleen and peripheral blood of 56 day-old chickens. In the presence of PATS, the specific LA for spleen cells was 50.1% and for peripheral blood, 49.4%. In the presence of PABS, the specific LA for spleen was 42.7% and for peripheral blood, 9.2%. These results then indicate that the splenic lymphocyte population is composed of Th and Bu antigen bearing lymphocyte populations while the lymphoid population of the peripheral blood is comprised of a large proportion of Th-antigen bearing cells, a large proportion of cells not recognized by either of the antisera, and a relatively small proportion of Bu antigen bearing cells.

The LA assay was then used to evaluate whether the PBLs bearing the Th antigen would react with PATS like Th cells. The results (represented in Figures 7a and b) show that PBLs react like Th cells when tested under similar conditions. The reaction at 38^0 C was slowed by increasing the volume of the reaction mixture without an equivalent increase in the surface available for adherence.

III. The Identification of Lymphocyte Antigens on PBLs That Participate in the GvH Reaction

Thus far, the usefulness of the antisera has been confined to an *in vitro* definition of surface antigens expressed on lymphoid cell populations of the thymus, bursa, spleen, and peripheral blood. The capacity of PATS to define one of the Th cell-mediated *in vivo* functions of Th-antigen bearing subpopulations was tested in two assay systems, the CAM pock and the splenomegaly assay. In the first assay system (the CAM pock test), allogeneic PBLs are inoculated onto the CAM of chick embryos, and 4 days later the number of pocks formed on the CAM measures the relative number of immunologically reactive cells remaining in the cell suspension. PBLs from $\underline{B}^{14}/\underline{B}^{14}$ donors (used as a convenient source of immunocompetent cells) were incubated in the presence or absence of PATS in the same manner described for the LA test. The nonadherent PBL population incubated in the absence or in the presence of PATS were inoculated onto the CAM of $\underline{B}^{2}/\underline{B}^{2}$ chick embryos. In some experiments, the adherent PBL population was collected by vigorous shaking and inoculated onto the CAM. In all the groups tested, 2-3x10^5 PBLs per chick embryo were inoculated onto the CAM. These treatments should result in a loss of GvH immunocompetence of the nonadherent population after incubation with PATS because of the selective loss of Th antigen bearing cells. Likewise, the GvH reactivity may be augmented by an increase in the concentration of Th antigen bearing cells of the adherent population.

The results of these experiments are summarized in Table III, experiments 1 through 7. In experiments 2, 3, 4, and 7 the GvH reactivity of the nonadherent PBL populations was significantly reduced after incubation with the antiserum compared to the control (GvH reactivity of PBLs incubated in the absence of antiserum). In these four experiments, GvH immunocompetence decreased by 91.5%, 63.9%, 78.4%, and 75.3%, respectively. However, the loss of adherent cells after antiserum treatment was not correlated with a loss of GvH immunocompetence within any single experiment or between experiments. In experiment 4, for example, PBLs were incubated with a 1:32, 1:64, 1:128, or 1:256 dilution of PATS. The GvH immunocompetence of these PBL populations was significantly reduced from the control group in only one of the antiserum treated groups (1:64) despite the fact that the same numbers of cells were used in the inocula after incubation with the antiserum. In experiments 1, 4, and 6 no significant loss of GvH immunocompetence was obtained in any of the groups of PBLs incubated with antiserum despite the adherence of a significant number of cells. In experiments 1 and 2, the GvH immunocompetence of the adherent PBL population was significantly reduced from the PBLs used in the control group.

In contrast to these inconsistent results, the *in vitro* pretreatment of PBLs with PATS had a profound and consistent effect upon the GvH reactivity of these cells measured in $\underline{B}^2/\underline{B}^2$ chick embryos using the splenomegaly assay (Figure 8). A complete suppression in the GvH reactivity of the PBLs was obtained after incubation with antiserum concentrations of PATS up to a dilution of 1:512. The mean spleen weight in $\log_{10}$ mg was an inverse function of the $\log_{10}$ concentration of antiserum used to treat the inoculum. The spleen weight decreased 33% for each tenfold increase in antibody between dilutions of 1:512 and 1:8192. At a dilution of 1:8192 and

1:16,384 the mean spleen weight equaled that of the positive control. The GvH reactivity of allogeneic PBLs measured in the splenomegaly assay decreased from a mean spleen weight of 102 mg in the control group (incubated *in vitro* without antiserum) to 9.3 mg in the experimental group of PBLs incubated *in vitro* for a period of 15 minutes with PATS at 22^0 C (Table IV). This loss of reactivity in the experimental group of PBLs did not vary with increasing time of incubation with the antiserum. In another experiment (Figure 9), the temperature of incubation was changed to 4^0 C and the results show that a consistent and complete suppression of the PBL, GvH reactivity was obtained by 30 minutes of incubation, *in vitro*, with the antisera. A change in the size of the culture tubes (17x100mm versus 12x75 mm) without a change in the volume of the incubation medium does not significantly alter the capacity of the antiserum to suppress the GvH reactivity of PBLs ($p>0.75$).

The CAM pock assay and the splenomegaly assay were also used to determine if PABS would alter the GvH reactivity of PBLs. The ability of PBLs to produce GvH pocks was not altered after incubation with PABS (Table III, experiments 8 and 9). On the other hand, the GvH reactivity of PBLs measured by the splenomegaly assay was severely suppressed (Figure 10) and the slope of the antibody dilution curve ($b=0.460$) was not different from that observed for PATS ($b=0.503$). Both PABS and PATS may then be recognizing cellular lymphocyte antigens common to PBLs, Th, and Bu cells.

Several experiments have been presented in Table V showing that incubation of PBLs with NPS has no effect upon the GvH reactivity of the inoculated cells. Also, washing the PBLs after in vitro pretreatment with either of the pheasant antisera did not significantly change the level of suppression of the GvH reaction (Table VI).

In the next series of experiments the PATS and PABS were absorbed with Th, Bu, or RB cells prior to the assay for GvH suppression. Two mls of a 1:16 dilution of PATS was absorbed once, twice, or three times with Th, Bu, or RB cells. Allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs were then incubated with the absorbed antiserum at 4^0 C for 30 minutes with a final antiserum dilution of 1:128, 1:256, or 1:512. The antiserum-treated PBLs were then inoculated into 12 day-old $\underline{B}^2/\underline{B}^2$ chick embryos at a concentration of $3x10^5$ cells per embryo. The results are presented in the form of histograms (Figures 11 and 12) for the mean spleen weights of the embryos inoculated with the different groups of antiserum treated PBLs. In both experiments, the results indicate that the antibody activity in the PATS, defined by the suppression of PBL, GvH reactivity in chick embryos, was removed by absorption of the antiserum with Th or Bu cells. Absorption of the antiserum with RBCs did not remove the antibody activity responsible for the suppression. Two similarly designed experiments (Figures 13 and 14) were conducted using PABS and absorbed PABS. The results show that both Bu and Th cells but not RBCs were capable of removing the PBL, GvH inhibitory activity of the antiserum. In summary, these experiments demonstrate the capacity of Th and Bu cells to remove the antibody activity, present in both PATS and PABS, that is responsible for the suppression of PBL, GvH reactivity in chick embryos. The antibody activity of these antisera are clearly directed against a lymphocyte antigen common to Th, Bu, and PBLs.

DISCUSSION

The results that have been presented in this chapter demonstrate that heteroantisera raised in pheasants against chicken thymus or bursa cells contain many types of species specific cell reactivities. This is not surprising in view of the long history that has preceeded this work regarding the raising of antilymphocyte serum and examination of its properties. One of the first accounts describing the preparation of antilymphocyte serum dates back to 1899, when Eli Metchnikoff raised in guinea pigs, anti-rat lymph node or spleen serum to study the cellular basis of inflammation.

Anti-Th or -Bu cell activity was identified in PATS or PABS on the basis of lymphocyte fixation to the surface of plastic or glass vessels after the addition of antiserum. The fixation of lymphocytes appears to represent a consistent, rapid, and useful method for the *in vitro* detection of Th and Bu cell antigens expressed on lymphoid cells comprising the primary and peripheral lymphoid tissue of the chicken. The organ distribution of Th and Bu cell types observed in the lymphocyte fixation test differs from that observed with immunofluorescent labeling tests reported by Hudson and Roitt 1973, and Wick *et al.*, 1975. The proportion of Th cells observed in the LA test is 10% lower than that reported using immunofluorescent labeling for both the peripheral blood and spleen. The proportion of Bu cells observed with the LA test is equal to that observed by others for the spleen and is lower by 14 to 33% for peripheral blood. The distribution of Bu cells in these lymphoid organs using lymphocyte fixation test equals the distribution frequency observed for these organs in studies using anti-immunoglobulin reagents to label Bu cells (Wick *et al.*, 1973). The Bu cell marker identified in the LA test with PABS, though, does not appear to be an anti-immunoglobulin marker since chicken immunoglobulin does not react with PABS in Ouchterlony tests.

Comparisons between the organ distribution of chicken Th and Bu cell types with those of mouse T and B cell types were made on the basis of the data presented for the chicken in Hudson and Roitt 1973, and Wick *et al.*, 1975, using anti-Th and anti-Bu serum reagents and for the mouse in Raff 1971, using anti-theta and anti-MBLA reagents. These comparisons reveal that: 1) the proportion of chicken peripheral blood Th cells is 12 to 14% lower than that observed for T cells in the blood of the mouse; 2) the proportion of chicken spleen Th cells is 25 to 27% higher than that observed for T cells in this organ of the mouse; 3) the proportion of chicken peripheral blood Bu cells is equivalent (33%) to that observed for B cells in the peripheral blood of the mouse; and 4) the proportion of chicken spleen Bu cells is 15 to 18% lower than that observed for B cells in this organ of the mouse. On the basis of this analysis the proportion of Th and Bu cell types of the chicken spleen is much closer to that observed for T and B cell types of the mouse lymph node (T=61%, B=28%) than that observed for any of the other lymphoid organ. The differences examined here may be real and may then reflect differences in the structuring of lymphoid tissue during the evolution of these two animal species. Alternatively, this difference may be attributed to the differences in methodology and antisera used to describe each system.

The selection of pheasants to raise heteroantisera against Th and Bu cell was based on the knowledge that mammalian antibody does not have the capacity to fix chicken complement. The incompatibility in the complement binding properties that exist between the mammalian and avian systems was described by Rice 1948, Rice and Crowson 1950, Orlans _et al_., 1962, and Gigli and Austin 1971. The use of mammalian heteroantisera for studying immune functions of Th- and Bu-derived lymphocytes by _in vivo_ depletion of these cells with antisera may seriously impair its specificity and effectiveness in these type of experiments since the _in vitro_ specificity of typing Th and Bu antigen-bearing lymphocytes require mammalian complement to complete the reactions of immune adherence or by cytolysis.

PATS and PABS as defined by lymphocyte fixation were tested for their usefulness in examining the role of Th or Bu antigen-bearing lymphocytes in the mediation of cellular immunocompetence against major histocompatibility antigens as measured in the GvH pock assay. The results of these experiments indicate that only pretreatment _in vitro_ with PATS will suppress the GvH reactivity of PBLs. However, this suppression is not directly correlated with the selective loss of Th antigen-bearing PBLs after _in vitro_ treatment with PATS. The suppression of antithymus serum on the PBL, GvH reactivity measured in the pock reaction has been reported by others (Potworowski _et al_., 1971; Rouse and Warner 1972; Wick _et al_., 1975). Each of these reports along with my own have inherent weaknesses either in the methodology or in the consistency of the results that tend to overshadow the conclusions drawn, ie. that this is a strictly Th-cell mediated reaction. For example, the CAM pock assay is known to be cell-dose dependent (Coppleson and Michie 1966; Longenecker _et al_., 1970). The reports of Potworowski _et al_., 1971, and Rouse and Warner 1972, are weak in methodology. Each of these reports indicate that pretreatment with rabbit-antithymocyte serum will selectively destroy Th cell antigen-bearing PBLs after the addition of guinea pig complement _in vitro_ but no attempt was made to readjust the cell concentration of the lymphocytes after incubation with the antiserum prior to using this as an inoculum in the GvH pock assay. Therefore, one would expect to see a loss in the GvH immunocompetence of the treated cells based upon the fact that a lower concentration of cells is being inoculated onto the CAM. Next, in the experiments using turkey antisera (Wick _et al_., 1975) and in my own experiments using pheasant antiserum the cell concentrations were readjusted after incubation with the antiserum but the decrease in lymphocyte GvH reactivity produced by these antisera was not consistent from experiment to experiment. Finally, none of the experiments reported thus far have demonstrated a dose response relationship between the concentration of antiserum and the loss of GvH immunocompetence.

The chicken lymphocyte antigen marker identified with PATS or PABS on the basis of the serum's ability to limit an increase in the host-spleen weight following inoculation of allogeneic PBLs into chick embryos differ in specificity from those identified in a similar assay system with rabbit-antithymocyte serum (Lydyard and Ivanyi 1971; Ivanyi and Lydyard 1972; Crone et al., 1972). In these reports the suppression of GvH splenomegaly following treatment of lymphocytes with antiserum was associated with a Th cell antigen marker. The determinations of specificity were based upon the differential potency of rabbit-antithymocyte serum as opposed to rabbit-antibursa serum to reduce the PBL, GvH reactivity and upon the absorptive capacity of only thymus cells to selectively remove this activity from the antiserum. The absorption studies presented in the results section indicate that thymus, bursa, peripheral blood lymphocytes but not erythrocytes have the capacity to absorb the antilymphocyte activity identified in both PATS and PABS. Similiar observations have been made in the mouse system. Antilymphocyte and antithymocyte serum treatment of lymphocytes *in vitro* reduced their potential to elicit *in vivo* a GvH reaction in neonates and F1 hybrids (Boak *et al.*, 1967; Brent *et al.*, 1967; Levey and Medawar 1967; Gallagher *et al.*, 1972; Richie *et al.*, 1975). The reduced potential of these lymphocytes is believed to be associated with: 1) the change in migration pattern, ie. the antiserum coated cells home to the liver and kidneys instead of to the spleen and lymph nodes (Martin and Miller 1967; Taub and Lance 1968; Richie *et al.*, 1975), 2) with the loss of their recirculation potential after the initial migration is complete (Richie *et al.*, 1975), and 3) with an active elimination of the cells in the recipient via opsonization mechanisms (Greaves *et al.*, 1969; Martin 1969; Lydyard and Ivanyi 1974).

The PBL, GvH reactivity can also be suppressed with alloantiserum directed against antigens associated with the *B* locus (Crone *et al.*, 1972). McBride *et al.*, 1966, used non-cytotoxic alloantisera (presumably anti-B) to define the origin of immunocompetent cells responsible for the serial transfer of GvH reactivity in 17 day-old embryos. More recently, anti-B sera have been used as a criterion for establishing the B genotypes of various donors whose cells were used for absorption (Simonsen 1973; 1975).

The use of anti-Th and anti-Bu reagents to define *in vivo* Th and Bu cell functions have thus far largely confirmed observations made earlier in ablation type experiments. These are: 1) the survival of skin allografts is prolonged after *in ovo* injections of duck antithymus serum (Toben and St Pierre 1972) and after *in vivo* injections of turkey antithymus serum before and during allogeneic skin grafting (Wick *et al.*, 1975); 2) the onset of induction of experimental allergic encephalomyelitis (produced experimentally by injection with myelin protein) is suppressed by injections with rabbit antithymus serum (Jankovic *et al.*, 1970a); 3) the onset of the development of spontaneous autoimmune thyroiditis in the Obese strain of chickens is suppressed by *in vivo* injections of turkey antibursa serum (Wick *et al.*, 1971); 5) the humoral immune response to *Brucella abortus* is suppressed by *in ovo* injections of duck antibursa serum (Toben and St Pierre 1972); and 6) the production of hemolysins against sheep erythrocytes in the *in vitro* direct and indirect plaque-forming cell assay is inhibited by treatment with rabbit antibursa serum (McArthur *et al.*, 1971; Potworowski *et al.*, 1971). The use of these reagents *in vivo* does not appear to affect cells of the thymus or bursa *per se* but does alter the distribution of peripheral Th or Bu antigen-bearing cells (Jankovic *et al.*, 1970b; Wick *et al.*, 1971; Toben and St Pierre 1972).

Chapter 2

The Ly-4 System of Antigens in the Chicken

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INTRODUCTION

Seven genetic loci excluding those associated with the H-2 locus of the more than 30 alloantigen systems characterized in the mouse code for lymphocyte alloantigens. Five of them, described previously (Ly-1, 2, 3, 5 and Thy-1), code for unique surface markers on thymus cells and subpopulations of circulating T lymphocytes and the other two loci (Ly-4 and PC-1) code for lymphoid alloantigens expressed on circulating B lymphocytes and on plasma cells.

In the chicken five genetic loci of the more than 14 alloantigen systems encode lymphocyte alloantigens. Three of them (A, B, C loci) also encode antigens found on chicken erythrocytes. B represents the major histocompatibility system of the chicken (Schierman and Nordskog 1961; Pazderka et al., 1975a) and is the counterpart of the H-2 system of the mouse. The B and C lymphocyte alloantigens were detected by agglutination (Schierman and Nordskog 1962) and the A and B lymphocyte alloantigens were detected by lymphocyte adherence in the presence of alloantisera (allofixation, Wong et al., 1972). The Th-1 locus codes for a pair of antithetical antigens expressed on thymus and thymus-derived chicken lymphocytes and the Bu-1 locus codes for a pair of antithetical antigens expressed on bursa and bursa-derived chicken lymphocytes (Gilmour et al., 1976). These alloantigens were detected by the complement-dependent lymphocyte cytotoxicity assay.

In this chapter, two new chicken lymphocyte alloantigens were identified by the reduction of GvH activity of lymphocytes following treatment with alloantisera prepared in two highly inbred lines of chickens (lines 6_1 and 7_2) identical at the A, B, and C loci (W. E. Briles, unpublished communication). This system of identification was used to establish the mode of inheritance of these alloantigens and their relationship to the B and Th-1 alloantigen systems.

MATERIALS AND METHODS

I. Animals

Parental line 6 subline 1 (L6) and line 7 subline 2 (L7) chickens were obtained as eggs from the East Lansing Poultry Research Laboratory, East Lansing, Michigan, and hatched in filtered air, positive pressure quarters free of the Marek's disease virus (MDV). These lines have been inbred since 1940, and have a coefficient of inbreeding in excess of 95% (Stone 1975). Parental chickens were used to produce F1 hybrids (L6xL7 crosses) and F1's were used to produce F2 progeny. Sixty-nine MDV-free F2 progeny and 32 infected with MDV were used to analyze the alloantigens specified by the *Ly*-4 locus. Eighteen MDV-free F2 progeny were used to study the relationship between the alloantigens determined by the *Ly*-4 and *Th*-1 loci. Eighteen MDV-free F2 progeny derived from matings between F1 hybrids of L6 and B^{14}/B^{14} chickens were used to study the relationship between the Ly-4 and the B antigens. The *B* alleles in these F2 progeny were determined as described in Pazderka *et al.*, 1975a. Seventy-five F3 progeny, produced from MDV-free F2 Ly-4.1 parents and from MDV-free F2 Ly-4.2 parents, were exposed to MDV at hatching. Line FP embryos (B^{15}/B^{21}) used in the GvH splenomegaly assay were obtained from Hyline International, Johnston, Iowa. Random bred embryos were obtained from the University of Alberta poultry farm, Edmonton, Alberta.

II. Antisera

Anti-line 6 peripheral blood (A6) antiserum was produced by i.v. injection of 3 to 4 mls of heparinized whole blood from MDV-free line 6 donors into MDV-free line 7 recipients. A reciprocal immunization using MDV-free line 7 whole blood was done in MDV-free line 6 recipients to produce anti-line 7 peripheral blood (A7) antiserum. Weekly immunizations were carried out during the first 2 months. These chickens were then rested for 1.5 months. Bimonthly immunizations were then continued for a period of 3.5 to 5 months. The immunized chickens were bled and the serum was collected and stored in the vapor phase of liquid nitrogen in 1 ml aliquots.

Anti-line 6 thymocyte (A6Th) antiserum and anti-line 7 thymocyte (A7Th) antiserum were kindly provided by Dr. D. Gilmour. Antisera were prepared by immunization of $L6_3$ with $L7_2$ and $L7_2$ with $L6_3$ thymus cells (Gilmour *et al.*, 1976).

III. Absorption of Antisera

Each antiserum was produced in a single recipient following multiple PBL cross-immunizations using several donors of the other line. Since these lines are not completely isogenic, a certain amount of non-specific antilymphocyte activity (directed against other antigen systems shared by some L6 and L7 chickens) was expected to be present in each of the antisera. This activity was detected using the splenomegaly inhibition assay. To remove this activity 1 ml of a 1:10 dilution of the appropriate serum was absorbed at room temperature with 60 to 80x10^6 PBLs (preparation described in the next section). This procedure removed completely all A6 reactivity from A7 serum and all A7 reactivity from A6 serum. The same absorption procedure was followed using varying concentrations of Th cells, Bu cells, PBLs, RBCs for the purpose of determining the types of cells that carry the alloantigen detected by the antisera.

IV. Preparation of PBLs and Erythrocytes

The preparation of PBLs and erythrocytes from whole blood were identical to that described in Materials and Methods of chapter I. To further enrich the lymphocyte concentration in the PBL fraction, these cells were incubated twice in a 60x15 mm petri dish at room temperature to remove contaminating granulocytes, thrombocytes, and monocytes (Wong *et al.*, 1972). The PBLs were usually prepared the day previous to embryo inoculation and stored overnight at 5° C.

V. Assay for Antilymphocyte Activity in A6 and A7 Serum

In 12x75 mm plastic tube, 5 to 10x10^6 PBL/ml from appropriate donors were incubated (for a period of 20 minutes at 4° C) with either a 1:50 dilution of A6 serum, a 1:50 to 1:250 dilution of A7 serum , or 1:50 dilution of normal chick serum (NS). The GvH reactivity of the cells from different treatment groups was then tested in the GvH splenomegaly assay as presented in chapter I. A volume of 2 ml was usually prepared for each group of embryo inoculations. The serum-cell mixture from each group was injected i.v. into groups of 10 to 13, 12 day-old chick embryos in a volume of 0.1 ml/embryo. Six days later the embryonic spleens were removed, weighed to the nearest mg, and the spleen weights were transformed to $\log_{10}$ (Longenecker et al. 1972). The $\log_{10}$ spleen weights in the different treatment groups from each donor were compared using Duncan's new multiple range test (Sokal and Rohlf 1969). Other statistical tests used to analyze the data (regression analysis, analysis of variance, goodness of fit X^2, RxC tests of association, Fisher's exact test) were as described in the texts of Steel and Torrie 1960, and Sokal and Rohlf, 1969.

VI. Assay for Antilymphocyte Activity in A6Th and A7Th Serum

The procedure for these tests was identical to that described in section V of this chapter except A6Th serum at a dilution of 1:60 was substituted for A6 serum and A7Th serum at a dilution of 1:60 to 1:240 was substituted for A7 serum.

VII. Marek's Disease

A) Assay for Marek's disease virus (MDV): Whole blood was obtained from parental L6 and L7 chickens and from F2, L6-type and L7-type chickens exposed to MDV by housing them in quarters containing MDV infected chickens. The blood samples were assayed according to Longenecker et al., 1975. Briefly, the blood samples were diluted 1:4 with saline. One-tenth ml of this blood was injected i.v. into groups of 6 to 12, 12-day $\underline{B}^2/\underline{B}^2$ chick embryos. The number of MDV induced lesions on the CAM of each embryo was counted five days later. The groups of embryos in these experiments were coded and the number of lesions counted in each group was done without knowing the identity of the donor.

B) Classification of chickens with respect to MD: F3 progeny, 18 of the L6-type and 57 of the L7-type from 7 different hatches were exposed to virulent MDV at hatching. These chickens were observed from 3 to 12 weeks after hatching and evidence of MD was recorded. Those that died during this period were autopsied in search of lymphoid tumors of MD. Those that survivied were terminated at the end of the observation period and were autopsied for the presence of MD lymphomas. These records were made without knowing the identity of the chickens with respect to the lymphocyte type.

RESULTS

I. The Identification of the Lymphocyte Determining Locus, Ly-4

A) The GvH reactivity of parental $L6_1$ and $L7_2$ PBLs: Whole blood obtained from parental $L6_1$ and $L7_2$ chickens was diluted with MEM and inoculated into 12-day old chick embryos at concentrations of 0.625 to 10×10^5 cells per inoculum. The cell dose, GvH response curves were nearly identical for both types of inocula (Figure 15). These curves show that the spleen weight increased 20% as the cell dose was doubled from 0.625×10^5 to 1.25×10^5 cells, increased 20% as the cell dose was increased from 1.25 to 5×10^5 cells (two doubling dilutions) and increased 50% as the cell dose was doubled from 5 to 10×10^5 cells per inoculum. At the intermediate concentrations (1.25, 2.5, 5×10^5 cells) the increase in spleen weight was nonsignificant. At these cell concentrations then, the spleen weights are least affected by fortuitous variations between experiments.

B) Effect of anti-line 6_1 (A6) and anti-line 7_2 (A7) sera on the GvH reactivity of L6 and L7 PBLs: L6 PBLs were incubated *in vitro* at 4° C for 20 minutes with varying concentrations of A6 serum ranging from a dilution of 1:50 (2% by final volume) to 1:1600 (0.06%). Similarly, L7 PBLs were incubated with varying concentrations of A7 serum. Each experiment was done twice. For both antisera, a concentration greater than 1:800 dilution (0.1%) reduced or limited the GvH splenomegaly of the inoculated lymphocytes (Figure 16). Each pair of 'like' serum treatments was pooled to obtain the two dilution curves. These curves for both serum treatments are sigmoidal in shape and the slopes refer to the linear middle segment of each curve, -0.700 for A6xL6 and -0.770 for A7xL7. The curves are parallel and the difference in the limitation of GvH splenomegaly between the two curves is attributable to a difference in potency. The least variation in the curves lie to the right on the graph in which the antisera exerts a maximal or near maximal effect on the lymphocytes. For the experiments that follow, a dilution of 1:50 for A6 and dilutions of 1:50 to 1:250 were used to reproduce a reduction in GvH splenomegaly of one third of the maxima for L6 and L7 PBLs. This is a difference of about 0.50 on a $\log_{10}$ scale.

This reduction was not altered by a change in the temperature of incubation (22° C instead of 4° C) nor by a change in both the temperature and time of incubation with the antisera (22° C instead of 4° C and 60 minutes instead of 20 minutes) (Table VII). Washing the cells after *in vitro* incubation with the antisera prior to inoculation did not alter the reduction of GvH splenomegaly (Table VII). The antisera did not agglutinate lymphocytes nor was it cytotoxic under the conditions decribed in these experiments. Incubation with antiserum did not alter the GvH immunocompetence of these lymphocytes measured in the CAM pock assay (Table VIII).

C) Specificity of these antisera: L6 PBLs were incubated with NS, A6 serum, or with A6 serum absorbed with L6 PBLs, L7 PBLs, L6 RBCs, or with L7 RBCs. Only absorption of A6 serum with L6 PBLs removed the limiting effect of A6 serum on the GvH spleen weight produced by L6 PBLs (Figure 17). The GvH splenomegaly of L7 PBLs in a similarly designed experiment was not limited by A7 serum absorbed with L7 PBLs but were limited by A7 serum and A7 serum absorbed with L7 RBCs, L6 PBLs, or with L6 RBCs (Figure 18). L7 PBLs were then incubated with NS, A7 serum, A7 serum absorbed with $2x10^8$ L7 PBLs, L7 Th cells, L7 Bu cells, and L7 RBCs or with A7 serum absorbed with $4x10^8$ L7 spleen cells, L7 Th cell, L7 Bu cells, and L7 RBCs. Only A7 serum absorbed with $2x10^8$ PBLs (Figure 19a) and only A7 serum absorbed with $4x10^8$ L7 spleen cells (Figure 19B) removed the limiting effect of A7 serum.

D) Limitation of GvH spleen weight by antisera: Parental L6, L7, and F1 PBLs were incubated with A6, A7, or NS _in vitro_ and inoculated intp chicken embryos. The results show that: 1) for L6 PBLs, A6 serum limited the GvH spleen weight while NS and A7 serum had no effect (Table IX, Tests 1 to 7); 2) for L7 PBLs, A7 serum limited the GvH spleen weight while NS and A6 serum had no effect (Table IX, Test 8 to 17); 3) for F1 PBLs, both A6 and A7 serum limited the GvH spleen weight while NS had no effect (Table IX, Tests 18 and 19). The GvH limitation by these antisera for each of the donors is expressed as the A7 serum spleen weight ($\log_{10}$ mg) minus the A6 serum spleen weight ($\log_{10}$ mg) (Table IX, column 4). In parental L6 this difference will be positive because of the absence of an effect with A7 serum and with parental L7 this difference will be negative because of the absence of an effect with A6 serum. In F1 chickens this difference is near zero because the two antisera have equal limiting effects.

The relationship between limiting and nonlimiting GvH spleen weight of L6 and L7 PBLs was examined by determining the correlation of the spleen weights for these treatments presented in Table IX. First, there is a very high correlation between the NS mean spleen weight and the mean spleen weight produced by L6 PBLs incubated with A7 serum or between the NS mean spleen weight and the mean spleen weight produced by L7 PBLs incubated with A6 serum (Figure 20, upper line, b = principal axis of correlation = 1.06, r=coefficient of correlation = 0.958, $p<0.001$). The mean embryo spleen weight for each donor whose PBLs are treated with NS does not differ significantly from the mean spleen weight of that donor's PBLs after treatment with the nonlimiting serum (ie. A7xL6 and A6xL7). Secondly, there is a very high correlation between the NS spleen weight or the nonlimiting spleen weight and the limited spleen weight produced by L6 PBLs incubated with A6 serum, the spleen weight produced by L7 PBLs incubated with A7 serum, and the spleen weights produced by F1 PBLs incubated with A6 and A7 serum (Figure 20, lower line, $b = 1.07$, $r = 0.813$, $p<0.001$).

The mean embryo spleen weight for each donor whose PBLs are incubated with NS or the nonlimiting serum differs significantly from the mean embryo spleen weight of that donor's PBLs after treatment with the limiting serum (ie. A6xL6, A7xL7, A6xF1, A7xF1). The correlations show that difference between the effects of A6 and A7 serum is effectively independent of fortuitous variations in the GvH spleen weight under the conditions of these tests. The differential limitation of GvH spleen weight, then, is a reliable measure of the correlation between the limiting and nonlimiting effects of antiserum on the spleen weight of PBLs obtained for each donor.

E) Classification of an F2 population: PBLs obtained from 101 F2 progeny produced in three separate hatches from the same breeding stock of F1, L6xL7 chickens were incubated with A6, A7, and NS and typed according to the differential limitation of GvH spleen weight into three classes, L6-type, L7-type, and F1-type. Division points were established between these classes before the F2 tests were done by dividing the differential limitation for L6 and L7 PBLs into three parts, one-quarter being assigned to the L6-type, one-half to the F1-type, and one quarter to the L7-type. The mean differences for L6, L7, and F1 chicken donors are 0.523±0.043, -0.517±0.032, and 0.139±0.050, respectively (Table IX). The first division point (0.263) therefore, separates those donors whose PBLs were limited only by A6 serum from those donors whose PBLs were limited by both A6 and A7 serum. Similarly, the second division point (-0.257) separates those donors whose PBLs are limited by only A7 serum from those whose PBLs are limited by both A6 and A7 serum. The classification of F2 donors on this basis resulted in the identification of the following frequency distributions of L6-, F1-, and L7-types for the first hatch-12:16:4, the second hatch-6:24:16, and the third hatch-4:12:7. The total distribution frequency then equaled 22 chickens which were classified as the L6-type, 52 as the F1-type, and 27 as the L7-type (Figure 21). This distribution, as well as the distributions obtained for each of the different hatches, does not differ from the Mendelian expectation for a system of two antigens encoded as alleles at a single genetic locus. This result affirms the validity of setting division points to classify the donor line type and also strongly implies that this is a new and distinctive locus for determining lymphocyte alloantigens. This locus is designated Ly-4 and the alleles are $\underline{Ly\text{-}4}^{\underline{a}}$ for L6-type chickens and $\underline{Ly\text{-}4}^{\underline{b}}$ for L7-type chickens.

The identification of three separate populations in an F2 population can also be examined using a probability graph. If there is one population the graph will show a straight line, if two a sigmoidal line, and if three a doubly inflected line. The probability graph presented in Figure 22 shows that this F2 population is really composed of three populations. A population made up of the data obtained for the L6 and L7 chickens (Table IX) has been superimposed on the graph to indicate the identity of the L6-type and L7-type subpopulations. The probability graph also indicates that the division between L6-type and F1-type, F2 chickens is too far to the right. This implies that the Mendelian distribution obtained in Figure 21 is most likely incorrect and should be adjusted by shifting the division points toward the center. This correction though is immaterial since the Mendelian distribution served the purpose of establishing the likelihood of finding three populations in the F2 which is the main point confirmed by the probability graph. None of these tests allows one to be definite about the individual genotype of an F2 chicken because this question can only be satisfied by testing their progeny.

II. The Ly-4 and Th-1 Antigen Systems

The alloantisera used to describe the Ly-4 system were produced by reciprocal immunizations of L6 and L7 chickens with whole blood. Other alloantisera have been produced by reciprocal immunizations between $L6_3$ and $L7_2$ with thymus or with bursa cells (Gilmour *et al.* 1976). These are known as anti-Th-1 (A6Th and A7Th) and anti-Bu-1 sera and the antigens belong to the systems known as Th-1 and Bu-1, respectively. A6Th and A7Th sera were used to limit the GvH spleen weight of embryos inoculated with PBLs obtained from 8 F2 L6-type (Ly-4.1/Ly-4.1) and 10 F2 L7-type (Ly-4.2/Ly-4.2) chickens to determine whether the Th-1 and Ly-4 antigen systems are identical. Parental L6 PBLs were limited only by A6Th serum and parental L7 PBLs were limited only by A7Th serum. Assuming that the Th-1 and Ly-4 are identical antigen systems, only A6Th serum should be able to limit the GvH spleen weight evoked by PBLs of the L6-type and only A7Th serum should be able to limit the GvH spleen weight evoked by L-7 type PBLs. The results of these tests in this selected F2 population are presented in Table X according to their putative genotypes based on the differential limitation of GvH spleen weight produced by the two pairs of antisera. These results show that 5 of the 8 Ly-4.1/Ly-4.1 chickens carried both Th-1 antigens and 4 of the 10 Ly-4.2/Ly-4.2 chickens carried both Th-1 antigens. These results indicate that these two antigen systems are not identical.

III. The Ly-4 and B Alloantigen Systems

A6 and A7 sera were used to limit the GvH spleen weight of embryos inoculated with PBLs obtained from 9 F2 chickens homozygous for the $\underline{B}^{2}$ allele and from 7 F2 chickens homozygous for the $\underline{B}^{14}$ allele to determine whether the Ly-4 and B antigen systems are identical. These progeny were hatched from F1's derived from parents of L6-type $\underline{B}^{2}/\underline{B}^{2}$ chickens and $\underline{B}^{14}/\underline{B}^{14}$ chickens. These F2 chickens were not specifically produced for this experiment but were used to test the question of whether these antigens are identical. If these antigen systems are identical, then A6 serum should limit the GvH spleen weight evoked by PBLs from the F2 chickens carrying the $\underline{B}^2$ allele. This was not the case, however. Four chickens carried both antigens of the Ly-4 system (Table XI) and the origin of the L7-type antigen identified in these F2 $\underline{B}^2/\underline{B}^2$ chickens could only have come from $\underline{B}^{14}$ parents. These results suggest that the two systems of antigens are not identical.

IV. Marek's Disease

Parental L6 and L7 chickens were infected with MDV at hatching. At weekly intervals these birds were bled and their lymphocytes were inoculated into veins of 12-day embryos of the same B genotype. The presence of the virus in the inoculum is revealed by the development of focal lesions on the CAM (Longenecker *et al.*, 1975). Blood cells from L6 chickens were less effective initiators of viral lesions than blood cells from L7 chickens after 4 weeks of exposure to MDV (Figure 23). F2 progeny of L6xL7 parents were tested in the same manner at ages of 4 to 7 weeks. The L6-type were less effective than the L7-type (Fisher's exact test, $P = 0.033$, Table XII). The mean number of virally induced *in ovo* lesions (VIO) per 0.1 ml of diluted blood obtained for the group of 10 MDV-infected L6-type chickens was 50% lower than the VIO lesions obtained after injections for the group of 4 MDV-infected L7-type chickens ($\log_{10}$ VIO±SE for L6-type was 0.702 ± 0.044 versus 1.022 ± 0.068 for the L7-type). This association was tested further by exposing L6-type and L7-type chickens of an F3 generation to MDV-infected chickens at hatching. Table XII identifies the number of F3 L6-type and L7-type chickens that survived during the 3 month exposure to MDV without visible signs of MD (row 1), those that died during the 3 month period without MD lymphomas (row 2), and those that developed MD (determined by the presence of lymphomas) and died (row 3). These classifications were mutually exclusive. These results indicate that chickens which carried the L6-type antigen were less susceptible to the development of MD lymphomas than those that carried the L7-type antigens (Fisher's exact test, $P = 0.029$). If these type of results can be verified in larger sample populations than those represented here, it could mean that the new antigen system may be associated with a genetic system which confers the Marek's insusceptibility to L6-type chickens and the susceptibility to L7 chickens.

DISCUSSION

Previous studies have shown that one of the antigens of the B system is associated with a relative resistance to Marek's disease (Hansen *er al.*, 1967; Brewer *et al.*, 1969; Briles and Oleson 1971; Hansen *et al.*, 1967; Longenecker *et al.*, 1976). MD is a naturally occurring, oncogenic disease of juvenile chickens for which the aetiological agent is the MDV. Its counterpart in humans may be the disease referred to as Burkitt's lymphoma. In subsequent studies this antigen (B^{21}) has been found in high frequency in survivors of several unrelated populations of chickens exposed to MD, in genetically selected MD resistant lines of chickens, and in representatives of the Red Jungle fowl, the progenitor of the chicken species (Pazderka *et al.*, 1975; Longenecker *et al.*, 1976). The probable adaptive value conferred by this gene system provided the basis for the search for other lymphocyte antigen systems which might be co-inherited with differences in susceptibility to MD. Lines 6 and 7 were utilized in this study on the basis of the following information: 1) these lines were selected, inbred, and maintained at the East Lansing Poultry Research Laboratory for differences in susceptibility to avian lymphoid leukosis (Waters 1954; Stone 1975); 2) they are homozygous for the same *B* allele (Pazderka *et al.*, 1975b); and 3) they differ markedly with respect to their susceptibility to MD, L6 is highly resistant and L7 is highly susceptible (Crittenden *et al.*, 1972; Stone 1975).

Repeated immunizations of each line with peripheral blood cells of the other line produced antisera reactive against peripheral blood cells. Initially, both the cytotoxicity and allofixation assays were used in attempts to type lymphocytes with the alloantisera but these assays were relatively insensitive. Lymphocytes of each line were then incubated with antisera *in vitro* and the GvH reactivity of these cells was then measured. Incubation with alloantisera had no effect on the number of CAM pocks evoked by PBLs but it had a profound effect on GvH splenomegaly. The differences in sensitivity of the two assays may mean that inhibition of the CAM assay requires the destruction of donor PBLs but inhibition of GvH splenomegaly may only require coating of the PBLs with antibody. Antibody-coated cells may fail to reach the embryo spleen by being trapped in other parts of the vascular system, an adherence phenomenon perhaps comparable to allofixation *in vitro*. The mechanism for this effect is not known. I concentrated on application of the inhibition of GvH splenomegaly as a method of typing lymphocyte antigens.

Antisera, A6 and A7, were used at concentrations of 1:50 to 1:250 which limited the GvH splenomegaly of an inoculum of $5x10^5$ cells to about one third of that obtained with normal serum. A6 did not limit the GvH reactivity of L7 PBLs nor did A7 limit the GvH reactivity of L6 PBLs. This effect then clearly shows the ability of the antisera to discriminate between lymphocytes of each type of donor line but this was not quite the case for the discrimination between the origin of the cell types, ie. Th- or Bu-type lymphoid cells. The serum activity was completely removed by absorption with PBLs and spleen lymphocytes, partially removed by absorption with thymus cells, and not removed with bursa cells or with erythrocytes. These results indicate that the antisera appear to identify antigens which may be shared by lymphocytes of the thymus and spleen but are absent from lymphocytes of the bursa and from erythrocytes. The antisera appear to be blocking the reactions of lymphocytes associated with the generation of a Th cell function, namely the GvH splenomegaly reaction. These results also indicate that this antigen system is independent, in expression from those of the A, B, and C systems whose antigens are found on erythrocytes as well as lymphocytes and is independent of those antigens associated with the Bu-1 system (Gilmour *et al* ., 1976) since bursa cells were incapable of removing the antilymphocyte activity in A7 serum. These results, though, do not show that the antigen system is genetically independent from the A, B, C, and Bu-1 antigen systems.

L6 and L7 chickens also differ markedly with respect to their cellular immunocompetence. L7 PBLs induce 3 times as many CAM pocks per unit volume of blood as L6 PBLs (Pazderka *et al*., 1975b). Initially, this was not regarded as an important factor in the selection of these lines but it became an important factor when the antisera were utilized to follow the inheritance of these new antigens in F2 progeny by the limitation of GvH splenomegaly. Studies of the inheritance of strong and weak GvH reactivity in the CAM pock assay have indicated that inheritance of the strength of the reaction follows the inheritance of the B antigen in F2 progeny (Longenecker *et al.*, 1972). As yet no evidence is available to show that differences in the GvH reactivity observed for L6 and L7 will be co-inherited with the B antigen since both are homozygous at the *B* locus for the same allele. In order to resolve the possible interaction of the different sensitivities in the GvH reactivity of F2 progeny, the GvH spleen weight obtained following treatment of donor cells with A6 serum was subtracted from the GvH spleen weight obtained following treatment with A7 serum. This difference proved to be much more consistent than comparisons of the absolute spleen weight after antiserum treatment and had the additional value of proving that the typing procedure does not depend on a narrow range of GvH reactions and are largely independent of the donor's intrinsic GvH competence.

The GvH spleen weight evoked by L6 PBLs was sensitive to A6 serum, that evoked by L7 PBLs was sensitive to A7 serum, and that evoked by F1 lymphocytes was sensitive to both antisera. It is not practical to attempt to distinguish L6 from F1 PBLs by A6 serum treatment or to distinguish L7 from F1 PBLs by A7 serum treatment. The easiest way to categorize the three types of PBLs is to subtract the result associated with the A6 serum sensitivity from that associated with the A7 serum sensitivity. With PBLs from donors of the F1 this difference is zero, of the L6 it is a large positive number, and of the L7 it is a large negative number. Thus, taking the difference between the two treatments with antisera which is the most consistent measure of the GvH spleen weight is also the most categorial.

A6 and A7 serum were used to categorize an F2 population as a test of the possibility that the differential effects on L6 PBLs and L7 PBLs are due to a pair of alleles. In order to do this it was necessary to set arbitrary limits for the three categories (L6-type, L7-type, and F1-type) prior to performing the tests. In the F2 population 22 were categorized as L6-type, 52 as F1-type, and 27 as L7-type. A probability graph however, confirmed the existence of three populations, but also showed that one of the pre-set divisions between categories is probably incorrect, ie. the division point separating L6-type F2 chickens from the F1 type is too far to the right. On the basis of these results it appears that L6 donors are homozygous for a gene designated *Ly-4*^{a}, that L7 donors are homozygous for its allele *Ly-4*^{b}, and that both alleles are transmitted in a conventional manner.

The antigens of this system appear to be independent of the antigens of the B and Th-1 systems (Tables X and XI). More importantly, they may be associated with a differential susceptibility to infection by MDV and a differential susceptibility to MD (Table XII). If so, the Ly-4 system of the chicken is the second lymphocyte antigen system to be associated with differential susceptibility to a herpesvirus induced lymphoma. The first was the B system of the chicken. The implication of the association of lymphocyte antigens as markers of differential susceptibility to a herpesvirus induced lymphoma should reinforce the search for such markers in the chicken and in humans and it may ultimately assist our understanding of the molecular basis of susceptibility and resistance.

Table I. The Effect of Substituting Normal Pheasant Serum for Normal Chicken Serum on the Lymphocyte Adherence of Thymus and Bursa Cells

Source of Antibody	Source of Fresh Serum			
	NCS		NPS	
	Th cells^{o}	Bu cells^{o}	Th cells^{o}	Bu cells^{o}
ATS^{1} 1:8^{+}	71.5 ± 2.2		83.2 ± 1.7*	
ABS^{2} 1:16^{+}		50.6 ± 3.4		63.9 ± 1.6^{ns}
MEM^{1}	6.3 ± 2.3		14.1 ± 2.1^{ns}	
MEM^{2}		15.6 ± 0.3		31.6 ± 1.4*

$^{+}$ Dilution of Antibody
^{o} Mean %LA±SE
^{1} Incubation Time-60 Min. at 22° C.
^{2} Incubation Time-120 Min. at 22° C.
* Differs at $p=0.01$, ns not significant

Table II. The Effect of the Addition of Normal Chicken Serum on the Lymphocyte Adherence of Thymus and Bursa Cells

Source of Antibody	Diluent			
	MEM		NCS	
	Th cells^o	Bu cells^o	Th cells^o	Bu cells^o
ATS^{1}1:64$^+$	37.0 ± 1.0		44.1 ± 6.1^{ns}	
ABS^{2}1:8$^+$		17.0 ± 2.9		50.3 ± 0.9*
ABS^{2}1:16$^+$		28.1 ± 2.5		69.8 ± 2.3*
NPS^{1}1:64$^+$	4.0 ± 0.4		6.3 ± 1.1^{ns}	
NPS^{2}1:8$^+$		32.7 ± 2.6		30.9 ± 2.2^{ns}

$^+$ Dilution

^{o} Mean %LA±SE

^{1} Incubation Time-60 Min. at 22° C.

^{2} Incubation Time-120 Min. at 22° C.

* Differs at $p=0.01$, ns not significant

Table III. The Effect of PATS and PABS on the GvH Immunocompetence of $\underline{B}^{14}/\underline{B}^{14}$ PBLs

Exp. No.	Source of Antiserum	Final Dilution(s) of Antiserum	Percent LA	% Reduction in CAM pocks[+]
1	ATS	1:64	47	A 62.8 NA 33.9
2	ATS	1:128	31	A 86.2 NA 91.5
3	ATS	1:32 to 1:256	53	NA 63.9[1]
4	ATS	1:32 to 1:256	59-51	NA 56.9[2]
5	ATS	1:16 to 1:128	35-29	NA 78.4[3]
6	ATS	1:8 to 1:32,768	59-0	NA 54.5[4]
7	ATS	1:64 to 1:512	59-21	NA 75.3[5]
8	ABS	1:8	5	A 0 NA 0
9	ABS	1:8 to 1:256	7-2	NA 53.1[2]

A-Adherent PBLs, NA-Non-adherent PBLs

[+] % Reduction=(Mean CAM pocks formed after incubation with antiserum ÷ mean CAM pocks formed after incubation with NCS)x100

[1] % Reduction at 1:128, 1:256, [2] Reduction at 1:64

[3] Reduction at 1:128, [4] Reduction at 1:128

[5] Reduction at all dilutions

Table IV. The Effect of Time of Incubation on the GvH Inhibition of Allogeneic B^{14}/B^{14} PBLs after Treatment with PATS at 22° C

Time of Incubation	Source of Antiserum	Mean Embryo Spleen Weight±SE ($\log_{10}$ mg)
0	NS	2.08±0.09 [10]
15	PATS	0.97±0.03 [9] *
30	PATS	1.02±0.03 [9] *
45	PATS	1.04±0.03 [9] *
60	PATS	1.02±0.04 [9] *

[ ] Number of embryos

* Differs from NS, $p<0.05$

Table V. The Effect of Normal Pheasant Serum on the GvH Reactivity of Allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs Measured in the Splenomegaly Assay of $\underline{B}^2/\underline{B}^2$ Chick Embryos

Source of Serum	Final Dilution of Serum	MSW ± SE ($\log_{10}$ mg)	Statistical[+] Significance
NCS	1:100	1.82 ± 0.04	--
NPS	1:16	1.87 ± 0.06	ns
NPS	1:64	1.82 ± 0.04	ns
NPS	1:2048	1.90 ± 0.05	ns
PATS	1:2048	1.15 ± 0.04	p=0.001
PABS	1:64	1.36 ± 0.04	p=0.001
NCS	1:100	1.95 ± 0.04	--
NPS	1:8	1.88 ± 0.04	ns
PATS	1:1024	1.20 ± 0.04	p=0.001
NCS	1:100	2.00 ± 0.06	--
NPS	1:8	1.88 ± 0.05	ns
PATS	1:1024	1.19 ± 0.08	p=0.001

MSW Mean Embryo Spleen Weight
[+] Tested in Analysis of Variance, Compared with NCS
ns Not Significant

Table VI. The Effect of Washing the PBLs after Treatment with PATS and PABS on the GvH Reaction

Source of Serum	Dilution of Serum	MSW ± SE ($\log_{10}$ mg)	Anova
NCS	100	1.79±0.05 [11]	$F_{2,26}=0.6$ $p>0.50$
NPS	8	1.82±0.07 [10]	
NPS (W)	8	1.89±0.07 [8]	
PATS	32	1.06±0.04 [10]	$F_{1,18}=0.25$
PATS (W)	32	1.09±0.04 [10]	$p>0.50$
PABS	8	0.97±0.03 [9]	$F_{1,17}=0.7$
PABS (W)	8	1.01±0.03 [10]	$p>0.25$

MSW Mean Embryo Spleen Weight
(W) Washed after Treatment with Serum
[ ] Number of Embryos

Table VII. The Effect of Changes in the Temperature and/or the Time of *In Vitro* Incubation with A6 and A7 Sera on the GvH Reactivity of L6 and L7 PBLs

Donor Type	Antiserum	Conditions of Incubation	MSW ± SE ($\log_{10}$ mg)
L7	NS	22°C 60 Min.	1.79±0.13(10)
	A7	22°C 60 Min.	1.21±0.06(12)*
	A7	4°C 20 Min.	1.27±0.08(10)*
L7	NS	22°C 60 Min.	2.17±0.10(10)
	A7	22°C 60 Min.	1.54±0.04(12)*
	A7	22°C 60 Min.[W]	1.58±0.04(12)*
	A7	4°C 20 Min.	1.50±0.03(13)*
	A7	4°C 20 Min.[W]	1.60±0.05(13)*
L6	NS	22°C 60 Min.	1.82±0.12(9)
	A6	22°C 60 Min.	1.22±0.05(11)*
	A6	4°C 20 Min.	1.06±0.04(11)*

MSW Mean Embryo Spleen Weight (Random Bred Embryos)

[W] PBLs Washed after Incubation

( ) Number of Embryos

* Differs at $p<0.05$ from the GvH Reactivity of NS Treated PBLs

Table VIII. The Effect of NS, A6, and A7 Sera on the GvH Reactivity of L6 and L7 PBLs in the CAM Pock Assay

Donor Type	Serum Treatment# NS	A6	A7
L7	0.89±0.16(13)	1.23±0.17(11)+	1.01±0.20(10)+
	0.81±0.14(13)	1.21±0.14(12)+	0.89±0.18(12)+
L6	0.62±0.13(12)	0.79±0.16(13)+	1.17±0.20(9) +
	0.46±0.16(11)	0.72±0.18(11)+	0.72±0.14(10)+

# Mean $\log_{10}$ Pock Count/Membrane

( ) Number of Embryos

+ Means Do Not Differ Significantly from NS Group

Table IX. Limitation of GvH Spleen Weight by Antisera.

		Serum			
		I	II	III	Difference:
		NS^{+}	A6^{+}	A7^{+}	III-II
Donor Cells	Test 1.	•1.942 ± 0.103(10)	▲1.535 ± 0.068(10)	△1.931 ± 0.081(10)	+0.396
	2.	•1.809 ± 0.128(9)	▲1.116 ± 0.050(11)	△1.824 ± 0.127(9)	+0.708
Parental	3.	•1.634 ± 0.099(10)	▲1.164 ± 0.026(13)	▲1.686 ± 0.077(12)	+0.522
L6	4.	•1.851 ± 0.077(10)	▲1.377 ± 0.091(10)	▲1.830 ± 0.097(11)	+0.453
	5.	●1.845 ± 0.048(11)	▲1.272 ± 0.030(12)	▲1.915 ± 0.092(10)	+0.643
	6.	●1.564 ± 0.060(11)	▲1.188 ± 0.043(11)	▲1.620 ± 0.042(11)	+0.432
	7.	●1.844 ± 0.063(8)	▲1.410 ± 0.063(9)	▲1.914 ± 0.066(10)	+0.504
	Mean	1.784	1.294	1.817	+0.523
	Test 8.	•1.822 ± 0.145(10)	▲1.693 ± 0.127(9)	△1.275 ± 0.076(10)	-0.418
	9.	•1.977 ± 0.125(12)	▲2.093 ± 0.118(11)	▲1.447 ± 0.103(11)	-0.646
	10.	•2.090 ± 0.077(8)	▲2.115 ± 0.088(8)	▲1.553 ± 0.108(10)	-0.562
	11.	•1.609 ± 0.077(13)	▲1.692 ± 0.106(11)	□1.221 ± 0.048(12)	-0.471
Parental	12.	•1.753 ± 0.108(11)	▲1.844 ± 0.101(12)	▲1.476 ± 0.071(13)	-0.368
L7	13.	●1.796 ± 0.027(10)	▲1.770 ± 0.050(10)	▲1.382 ± 0.023(10)	-0.388
	14.	●2.207 ± 0.028(10)	▲2.199 ± 0.039(10)	▲1.606 ± 0.048(11)	-0.593
	15.	●2.276 ± 0.046(10)	▲2.237 ± 0.044(11)	▲1.584 ± 0.056(12)	-0.653
	16.	●1.905 ± 0.049(9)	▲1.907 ± 0.046(11)	▲1.357 ± 0.044(11)	-0.549
	17.	●2.203 ± 0.050(11)	▲2.205 ± 0.065(10)	▲1.682 ± 0.057(12)	-0.523
	Mean	1.964	1.905	1.458	-0.517
F1	Test 18.	•2.089 ± 0.155(9)	▲1.604 ± 0.175(8)	△1.794 ± 0.124(9)	+0.192
L6 x L7	19.	•1.933 ± 0.077(8)	▲1.521 ± 0.131(9)	△1.610 ± 0.119(10)	+0.089
	Mean	1.817	1.562	1.702	+0.138

+ Measurement of mean spleen wt ($\log_{10}$ mg) ± SE

• GVHR measured in outbred chick embryos

● GVHR measured in B^{15}/B^{21} chick embryos

— differs at p = .01 from normal serum group

-- differs at p = .05 from normal serum group

▲ 1:50 Antiserum concentration

△ 1:100 Antiserum concentration

□ 1:250 Antiserum concentration

Analysis of Variance	L6	L7	F1
a) for tests	$F_{6,197}=9.197^{***}$	$F_{9,288}=16.9^{***}$	$F_{1,47}=2.22^{ns}$
b) for sera	$F_{2,197}=111.4^{***}$	$F_{2,288}=137.7^{***}$	$F_{2,47}=5.93^{**}$
c) for interaction of tests and treatments	$F_{12,197}=0.445^{ns}$	$F_{18,288}=1.16^{ns}$	$F_{2,47}=0.00^{ns}$

ns nonsignificant; ** $0.001 > p > 0.005$; *** $p < 0.001$

Table X. Distribution of *Th*-1 Genotypes in F2, *Ly*-4 Homozygous Chickens

Genotypes

Ly-4

Genotypes *Th*-1	a/a	b/b	Totals
a/a	3	1	4
a/b	5	4	9
b/b	0	5	5
Totals	8	10	18

Ly-4^{a} corresponds to Ly-4.1/Ly-4.1 Phenotype
Ly-4^{b} corresponds to Ly-4.2/Ly-4.2 Phenotype
Th-1^{a} corresponds to Th-1.1/Th-1.1 Phenotype
Th-1^{b} corresponds to Th-1.2/Th-1.2 Phenotype

Table XI. Distribution of *Ly*-4 Genotypes in F2, *B*^{2} and *B*^{14} Homozygotes

		Genotypes *B*		
		2/2	14/14	Totals
Genotypes *Ly*-4	a/a	3	1	4
	a/b	4	3	7
	b/b	2	3	5
	Totals	9	7	16

Table XII. Marek's Disease Susceptibility of L6-Type and L7-Type F2 and F3 Chickens

VIO CAM Lesions	Ly-4.1/Ly-4.1	Ly-4.2/Ly-4.2	
Less Than 20 Per 0.1 ml Blood	10	1	F2
More Than 20 Per 0.1 ml Blood	1	3	

Fisher's Exact Test, P = 0.033

Survived without Lymphoma	8	5	F3
Death	4	14	
Death with Lymphoma	6	32	

Fisher's Exact Test: For Death, P = 0.003
For Lymphoma, P = 0.029

X^2 For Death and Lymphoma, 10.6, $p<0.005$

Figure 1. The lymphocyte adherence (LA) of thymus cells measured 60 minutes after incubation with varying concentrations of PATS *in vitro* at 22° C. Four different experiments are presented and each point is the mean of two tests. The upper horizontal dashed line represents the mean LA of Bu cells incubated with the varying concentrations of PATS. The lower horizontal solid line represents the mean LA of Th cells incubated with a 1:8 dilution of NCS.

Figure 2. The lymphocyte adherence (LA) of bursa cells measured 120 minutes after incubation with varying concentrations of PABS *in vitro* at 22° C. Two different experiments are presented and each point is the mean of two tests. The upper solid horizontal line represents the mean LA of Bu cells incubated with a 1:8 dilution of NCS. The lower horizontal dashed line represents the mean LA of Th cells incubated with the varying concentrations of PABS.

Figure 1

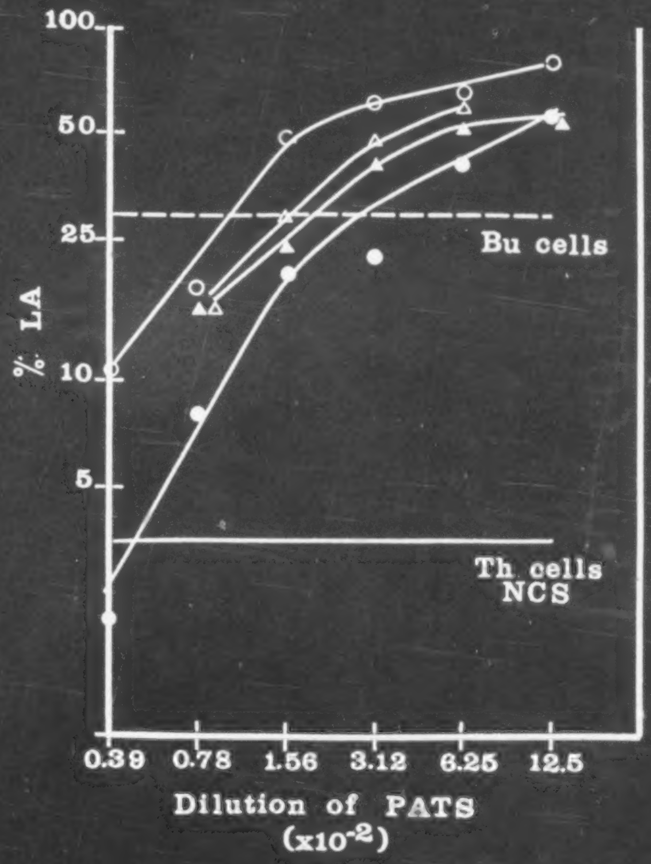


Figure 2

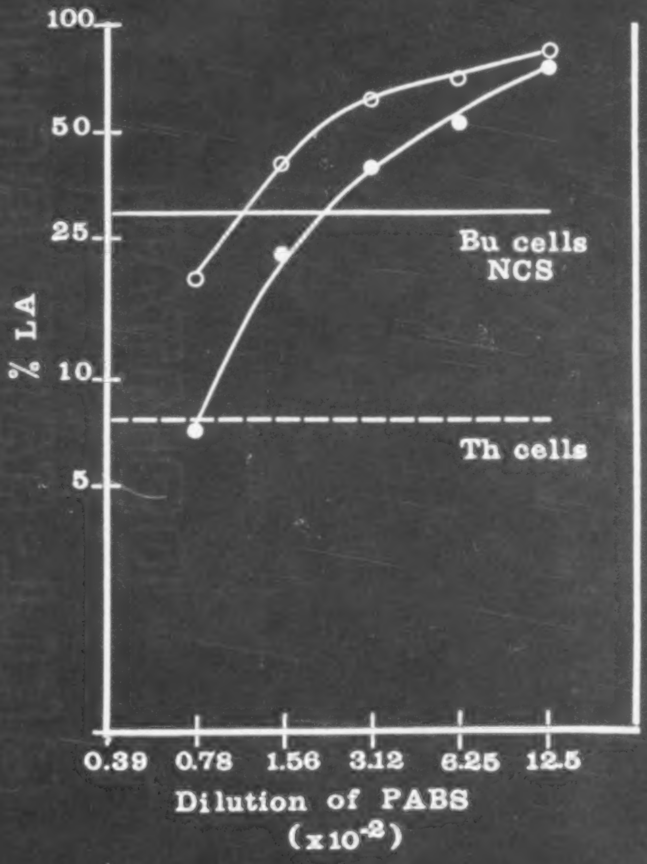


Figure 3. The effect of varying time of incubation on the LA of Th cells incubated with PATS and NCS at 22° C. The slope of the regression line (b) is linear ($p<0.001$) between the intervals of 30 and 120 minutes of incubation. The LA of Th cells differed significantly with time of incubation ($p<0.001$).

Figure 4. The effect of varying time of incubation on the LA of Bu cells incubated with PABS and NCS at 22° C. Each point represents the mean of three tests for LA. The slope of the regression line (b) is linear ($p<0.001$) between time intervals of 15 to 120 minutes. The deviations from linearity are nonsignificant ($p>0.50$). The percent LA of Bu cells differed significantly with times of incubation ($p<0.001$).

Figure 3

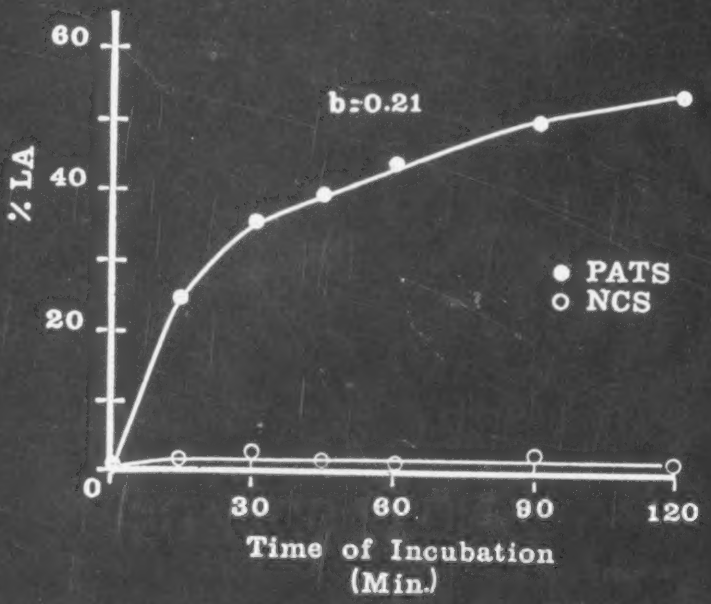


Figure 4

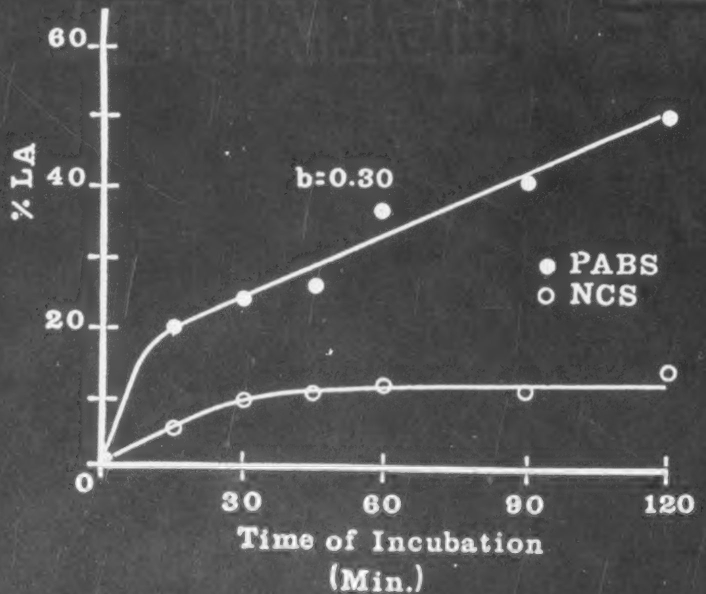


Figure 5a. The effect of varying time and temperature of incubation the on LA of Th cells incubated with a 1:32 dilution of PATS. Each point represents two tests for LA of thymus cells incubated at 22° C and 38° C. The LA of Th cells varied significantly with time ($F_{5,30} = 65.6$, $p<0.001$) and the regression lines are linear. The LA of Th cells incubated at 38° C was significantly higher than those incubated at 22° C.

Figure 5b. The effect of varying time and temperature of incubation on the LA of Th cells incubated with a 1:64 dilution of PATS. The LA of Th cells varied significantly with time ($p<0.001$) and the regression lines are linear. The LA of Th cells was higher at 38° C than at 22° C.

Figure 5a

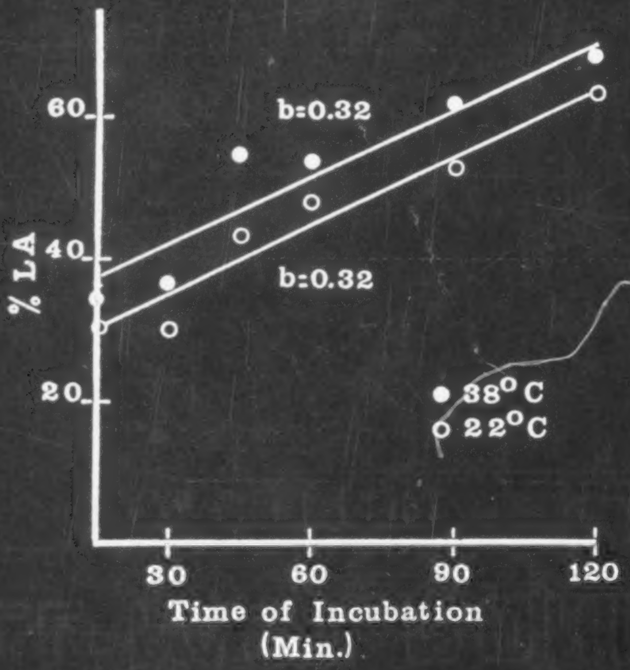


Figure 5b

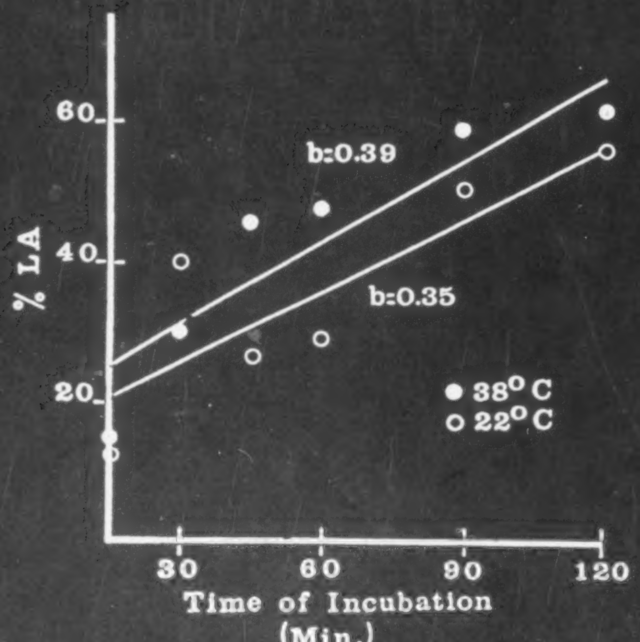


Figure 6. The distribution of Th and Bu antigens on lymphocytes obtained from the thymus and bursa of newly hatched to 56 day-old chicks. Each point represents the mean LA of tests from two donors. The distribution of Th antigens on lymphocytes from the thymus and bursa are represented on the LA of Th cells incubated with PATS (○) and Bu cells incubated with PATS (▲) for 60 minutes at 22^{0} C. The distribution of Bu antigens on lymphocytes from the bursa and thymus are represented by the LA of Bu cells incubated with PABS (△) and Th cells incubated with PABS (●) for 120 minutes at 22^{0} C.

Figure 6

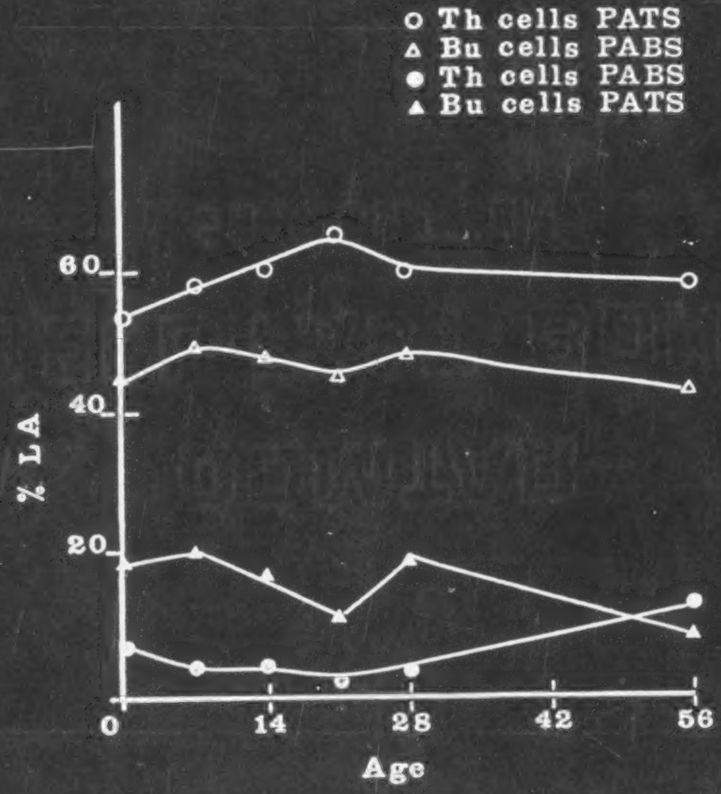

○ Th cells PATS
△ Bu cells PABS
● Th cells PABS
▲ Bu cells PATS
60
40
20
% LA
0
14
28
42
56
Age

Figure 7a. The effect of varying time of incubation and size of the reaction tubes on the LA of PBLs incubated with PATS at 38° C. The concentration of cells, serum, antiserum, and medium added to the large tubes (17x100 mm) was 5 times the amount added to the small tubes. Each point represents the mean of three tests for groups incubated in large plastic tubes with a total volume of 1 ml and those incubated in small glass tubes with a total volume of 0.20 ml.

Figure 7b. The effect of varying time of incubation and size of the reaction tubes on the LA of PBLs incubated with PATS at 22° C. The tests were performed in exactly the same manner described above.

Figure 7a

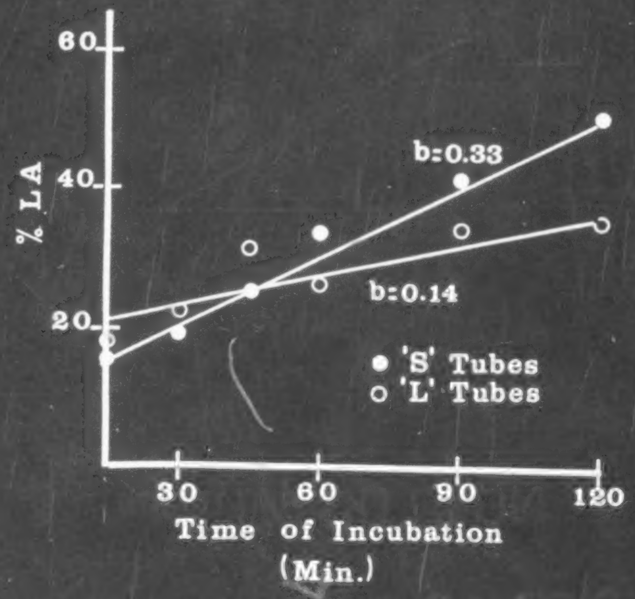


Figure 7b

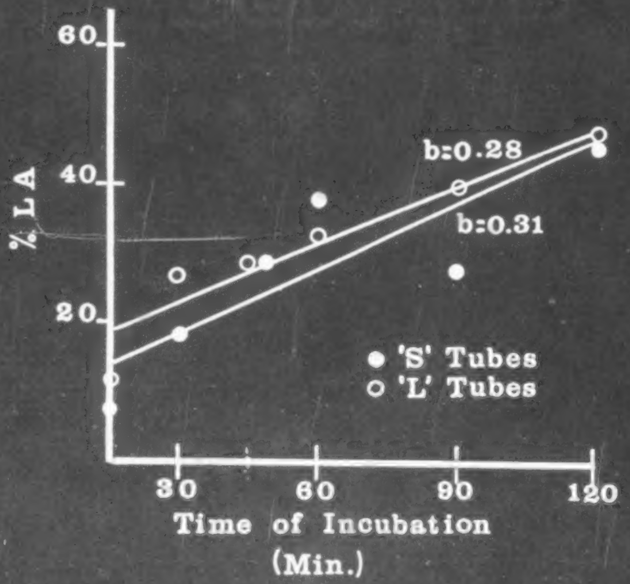


Figure 8. The GvH reactivity of allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs after incubation with PATS and NS measured in the splenomegaly assay of $\underline{B}^{2}/\underline{B}^{2}$ chick embryos. Each point represents the mean spleen weight (MSW) of 8 to 12, chick embryos inoculated with $3x10^{5}$ PBLs after incubation with antiserum in large plastic tubes in a volume of 1 ml. The upper solid and dashed horizontal lines represent the GvH reactivity (MSW±SE) of PBLs after incubation with PS. The lower solid and dashed lines represent the MSW±SE of uninoculated chick embryos. The dilution curve is linear ($p<0.001$) and nonsignificant for deviations from linearity ($p>0.025$) at the PATS concentrations of 0.0097 to 0.00012.

Figure 8

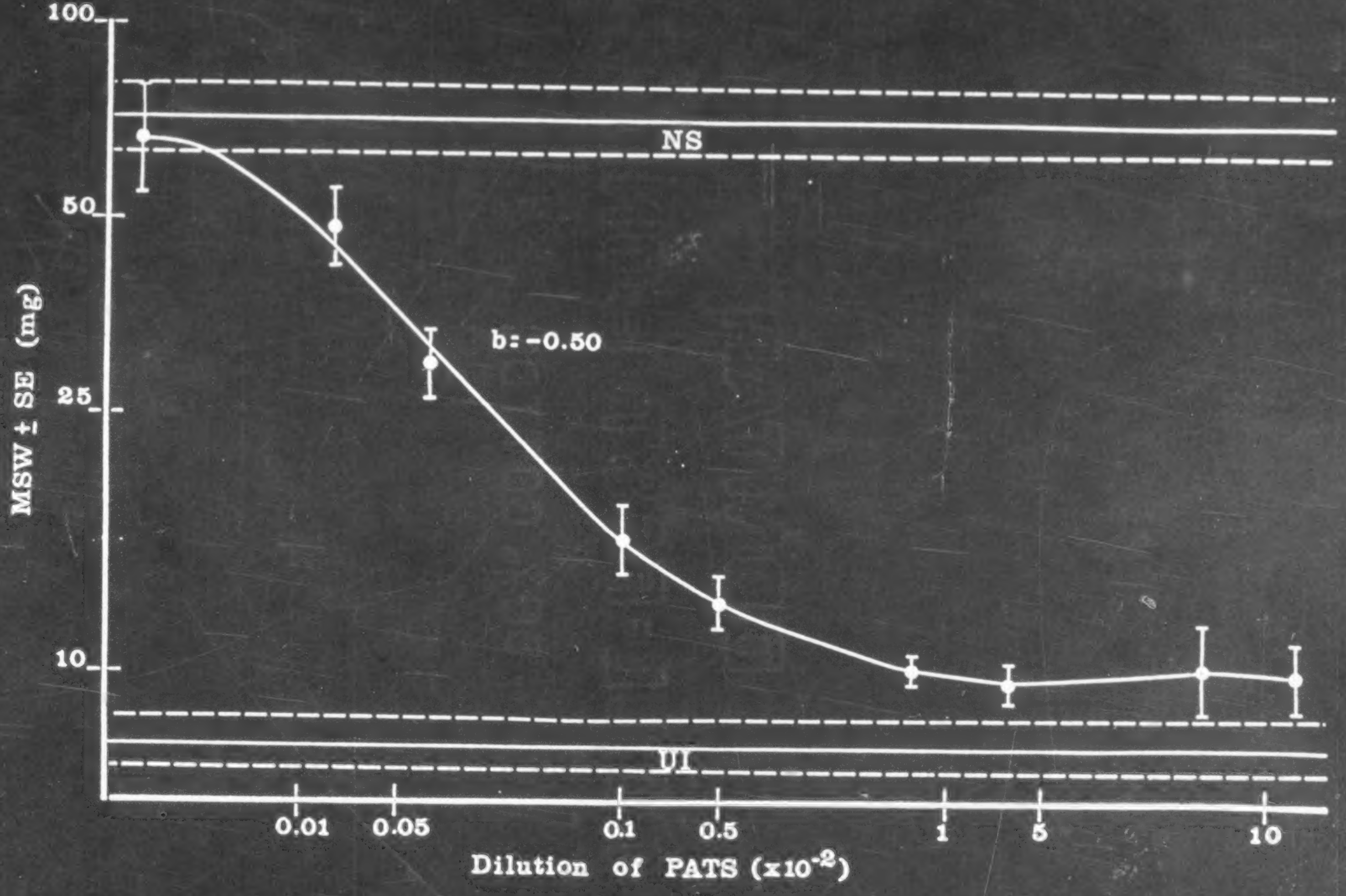

MSW ± SE (mg)
100
50
25
10
NS
b: -0.50
UI
0.01
0.05
0.1
0.5
1
5
10
Dilution of PATS (x10^{-2})

Figure 9. The GvH reactivity of $\underline{B}^{14}/\underline{B}^{14}$ PBLs after incubation with PATS at varying time intervals in large and small culture tubes. The closed circles represent the GvH reactivity of $3\text{x}10^{5}$ PBLs after 15, 30, 45, and 60 minutes incubation with the antiserum in small glass tubes (S) with a volume of 0.2 ml and at a temperature of incubation of 4^{0} C. The open circles represent the GvH reactivity of $3\text{x}10^{5}$ PBLs after 15, 30, 45, and 60 minutes incubation with the antisera in large plastic tubes (L) with a volume of 1 ml and at a temperature of incubation of 4^{0} C. The points connected with the solid line were the results obtained after incubation with a 1:128 dilution of PATS and those connected by the broken lines with a 1:1024 dilution of PATS. The upper solid horizontal line represents the MSW of PBLs treated with NS (control group). The loss of GvH reactivity after PATS treatment was not altered by a change of the tube's size ($p>0.75$) but was significantly altered by time of incubation (15 minutes was not as effective as 30 minutes, $p<0.001$) and by the dilution of PATS used (1:128 reduced the GvH reactivity more than 1:1024, $p<0.001$).

Figure 9

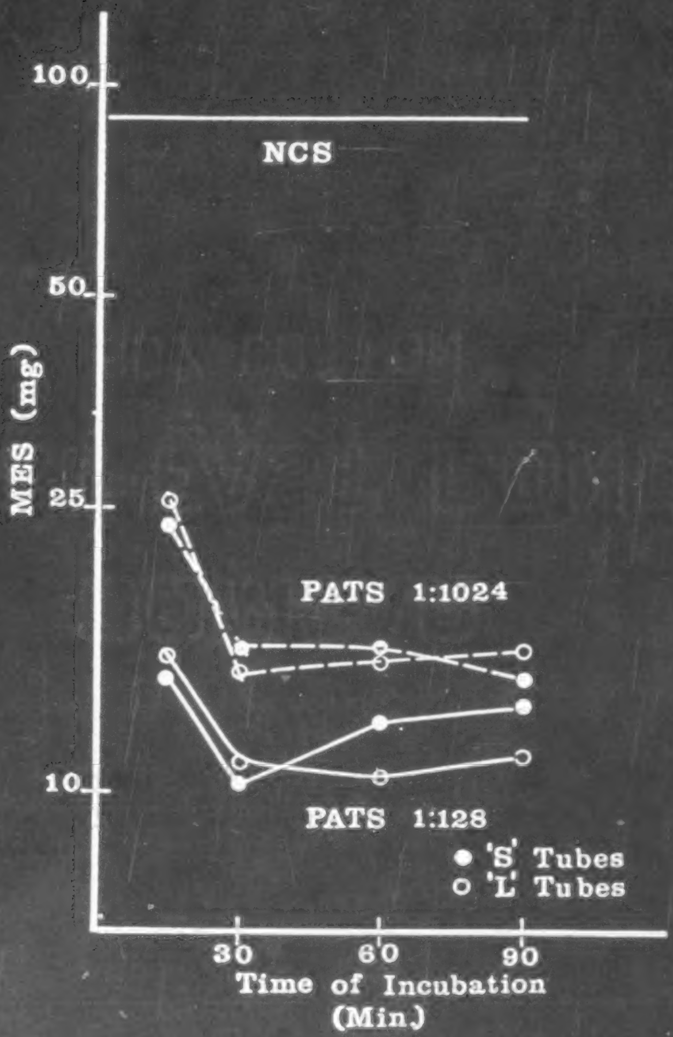

100
50
25
10
MES (mg)
NCS
PATS 1:1024
PATS 1:128
● 'S' Tubes
○ 'L' Tubes
30
60
90
Time of Incubation
(Min.)

Figure 10. The GvH reactivity of allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs after incubation with PABS and NS measured in the splenomegaly assay of $\underline{B}^{2}/\underline{B}^{2}$ chick embryos. The closed circles represent the MSW of 8 to 12 chick embryos inoculated with $3x10^5$ PBLs after incubation with antiserum in large glass tubes in a volume of 1 ml. The upper solid and dashed horizontal lines represent the GvH reactivity (MSW±SE) of PBLs after incubation with NS. The lower solid and dashed horizontal lines represent the MSW±SE of uninoculated chick embryos. The dilution curve is linear ($p<0.001$) and nonsignificant for deviations from linearity ($p>0.10$) at the PABS concentrations from 0.062 to 0.00049.

Figure 10

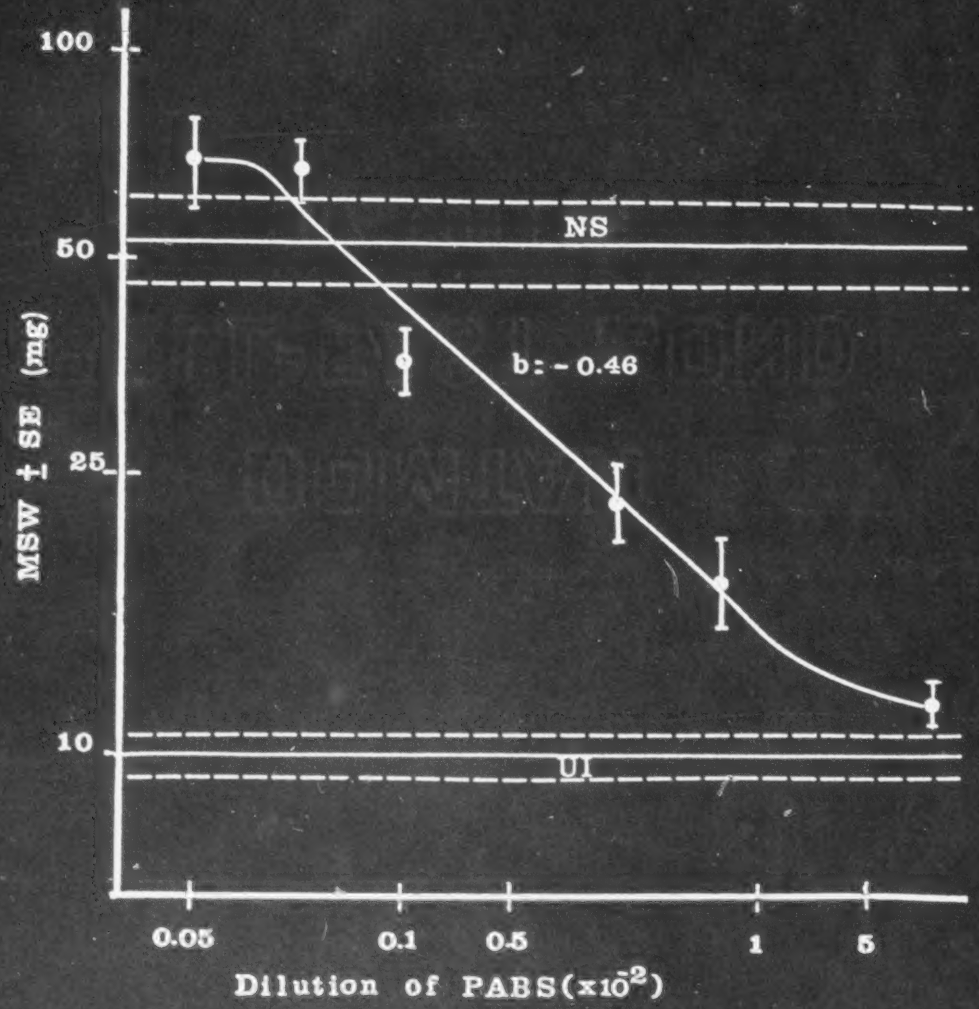

100
50
25
10
MSW ± SE (mg)
NS
UI
b: - 0.46
0.05
0.1
0.5
1
5
Dilution of PABS(x10^{-2})

Figure 11. Experiment 1. The GvH reactivity of allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs after incubation with PATS absorbed with $\underline{B}^{2}/\underline{B}^{2}$ Th, Bu, or RB cells measured in the splenomegaly assay of $\underline{B}^{2}/\underline{B}^{2}$ chick embryos. The bars of the histogram represent the MSW+SE of chick embryos inoculated with 3×10^5 PBLs after incubation with absorbed PATS in the concentrations of 1:128 (A), 1:256 (B), or 1:512 (C) dilution. The incubation of PBLs with the absorbed antiserum was performed at 4° C for 30 minutes. Six types of PATS were used: PATS unabsorbed (UA) and PATS absorbed once (I) or twice (II) with either Th, Bu, or RB cells. The upper solid and dashed horizontal line represent the MSW+SE of chick embryos inoculated with 3×10^5 PBLs incubated in the absence of antiserum. UI is the MSW+SE of uninoculated chick embryos. The solid square indicates a significant increase of the MSW ($p=0.05$) over that of the MSW of chick embryos inoculated with PBLs incubated with PATS absorbed twice with RB cells.

Figure 11

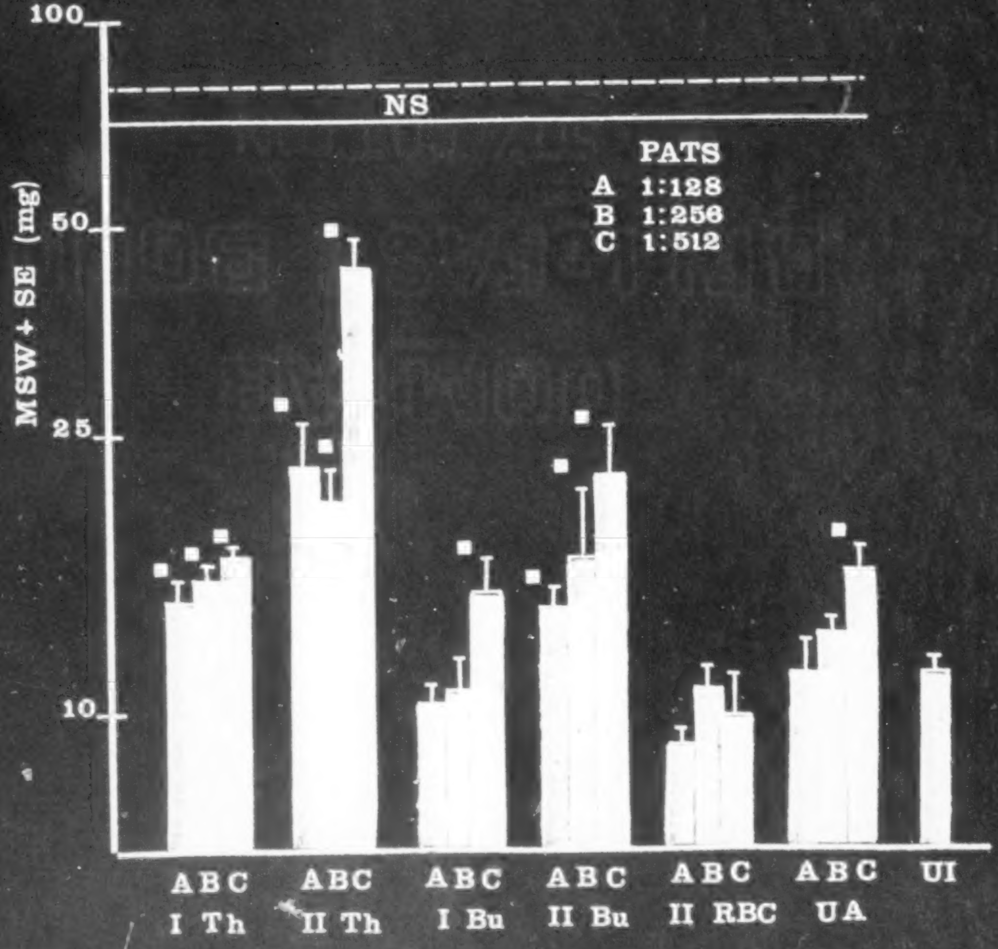

100
50
25
10
MSW + SE (mg)
NS
PATS
A 1:128
B 1:256
C 1:512
A B C
I Th
A B C
II Th
A B C
I Bu
A B C
II Bu
A B C
II RBC
A B C
UA
UI

Figure 12. Experiment 2. The GvH reactivity of allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs after incubation with PATS absorbed with $\underline{B}^{2}/\underline{B}^{2}$ Th, Bu, or RB cells measured in the splenomegaly assay of $\underline{B}^{2}/\underline{B}^{2}$ chick embryos. The bars of the histogram represent the MSW+SE of chick embryos inoculated with $3x10^{5}$ PBLS after incubation with absorbed PATS in the concentrations of 1:128 (A), 1:256 (B), or 1:512 (C) dilution. The incubation of PBLs with the antiserum was performed at 4^{0} C for 30 minutes. Six types of absorbed PATS were used: PATS was absorbed once (I), twice (II), or three times (III) with either Th, Bu, or RB cells. The upper solid and dashed horizontal line represent the MSW+SE of chick embryos inoculated with $3x10^{5}$ PBLs incubated in the absence of antiserum. UI is the MSW+SE of uninoculated chick embryos. The solid square indicates a significant increase of the MSW ($p=0.05$) over that of the MSW of chick embryos inoculated with PBLs incubated with PATS absorbed twice with RBCs.

Figure 12

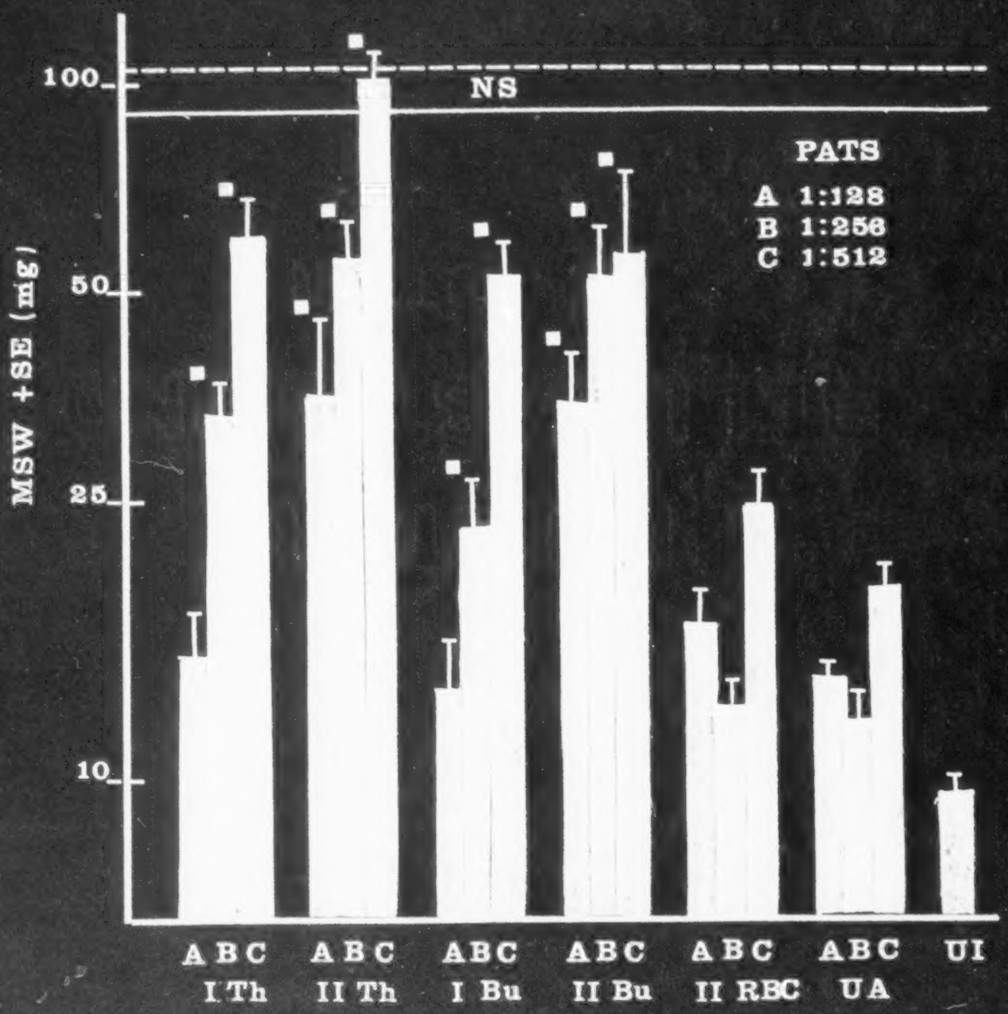

MSW +SE (mg)
100
50
25
10
NS
PATS
A 1:128
B 1:256
C 1:512
A B C
I Th
A B C
II Th
A B C
I Bu
A B C
II Bu
A B C
II RBC
A B C
UA
UI

Figure 13. Experiment 1. The GvH reactivity of allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs after incubation with PABS absorbed with $\underline{B}^{2}/\underline{B}^{2}$ Th, Bu, or RB cells measured in the splenomegaly assay of $\underline{B}^{2}/\underline{B}^{2}$ chick embryos. The bars of the histogram represent the MSW±SE of chick embryos inoculated with 3×10^{5} PBLs after incubation with absorbed PABS in the concentrations of 1:32 (A), 1:128 (B), and 1:256 (C) dilution. The incubation of PBLs with the absorbed antiserum was performed at 4^{0} C for 30 minutes. Three types of PABS were used: PABS absorbed twice (II) with either Bu, Th, or RB cells. The upper solid and dashed horizontal line represents the MSW+SE of chick embryos inoculated with 3×10^{5} PBLs incubated in the absence of antiserum. UI is the MSW+SE of the uninoculated chick embryos. The solid square indicates a significant increase of the MSW ($p=0.05$) over that of the MSW of chick embryos inoculated with PBLs incubated with PABS absorbed twice with RBCs.

Figure 13

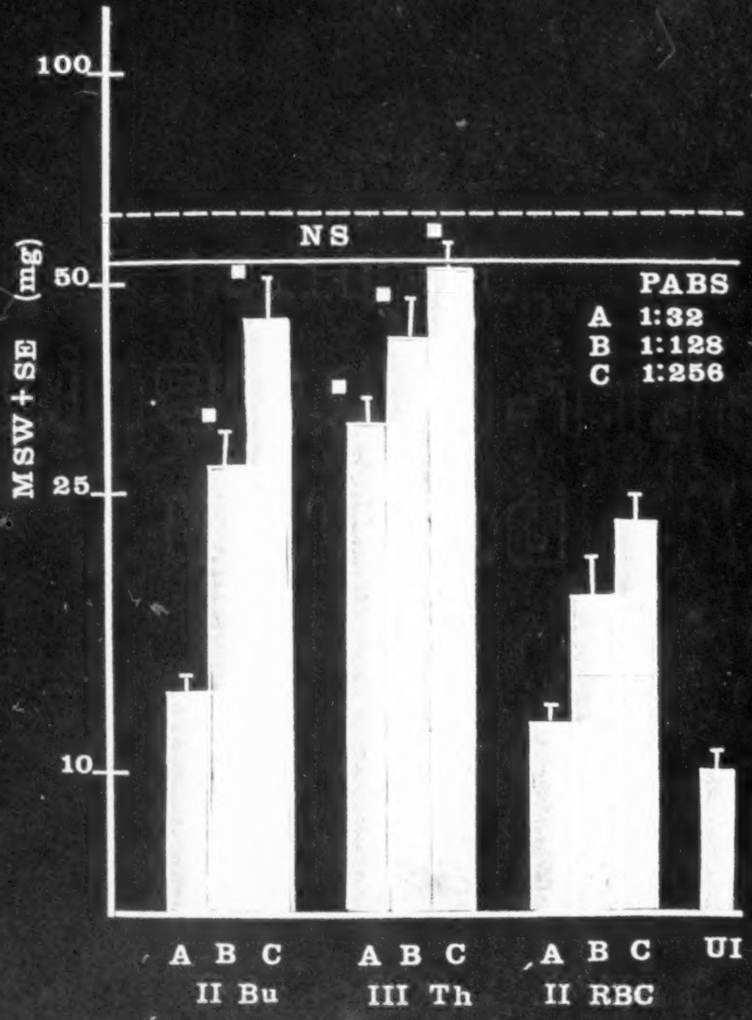

100
50
25
10
MSW + SE (mg)
NS
PABS
A 1:32
B 1:128
C 1:256
A B C
II Bu
A B C
III Th
A B C
II RBC
UI

Figure 14. Experiment 2. The GvH reactivity of allogeneic $\underline{B}^{14}/\underline{B}^{14}$ PBLs after incubation with PABS absorbed with $\underline{B}^{2}/\underline{B}^{2}$ Th, Bu, or Rb cells measured in the splenomegaly assay of $\underline{B}^{2}/\underline{B}^{2}$ chick embryos. The bars of the histogram represent the MSW+SE of chick embryos inoculated with 3×10^{5} PBLs after incubation with absorbed PABS in the concentrations of 1:64 (A), 1:128 (B), and 1:256 (C) dilution. The incubation of PBLs with the absorbed antiserum was performed at 4^{0} C for 30 minutes. Five types of PABS were used: PABS absorbed once (I) or twice (II) with either Bu, Th, or RB cells. The upper solid and dashed horizontal line represent the MSW+SE of chick embryos inoculated with 3×10^{5} PBLs incubated in the absence of antiserum. UI is the MSW+SE of uninoculated chick embryos. The solid square indicates a significant increase of the MSW ($p=0.05$) over that of the MSW of chick embryos inoculated with PBLs incubated with PABS absorbed twice with RB cells.

Figure 14

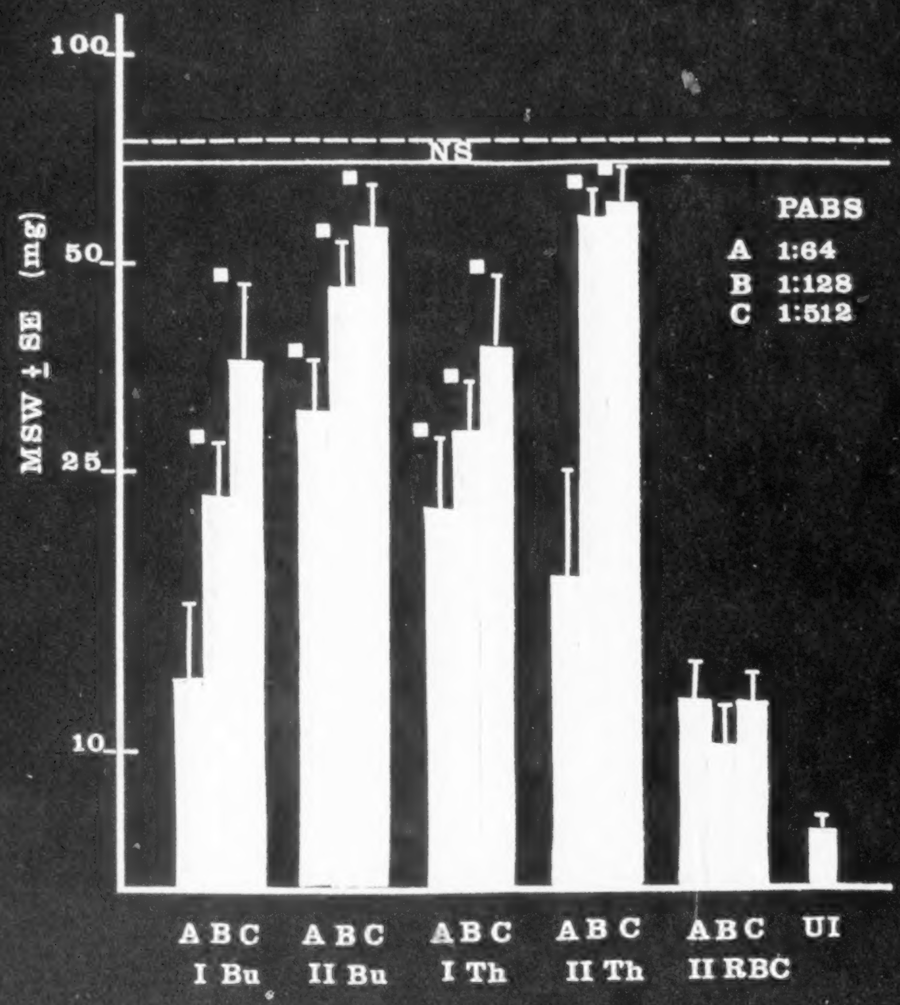

100
50
25
10
MSW ± SE (mg)
NS
PABS
A 1:64
B 1:128
C 1:512
A B C
I Bu
A B C
II Bu
A B C
I Th
A B C
II Th
A B C
II RBC
UI

Figure 15. The GvH reactivity of inocula containing L6 or L7 PBLs. Dilutions of whole blood were injected into 12-day chick embryos (random bred). This produced an increase in the mean embryo spleen weight (MSW) which was directly related to the number of cells inoculated. Both types of PBLs gave a response nearly identical to one another and they are represented in a single curve. The middle segment of the curve is linear ($p<0.001$) and the slope refers to this region.

Figure 15

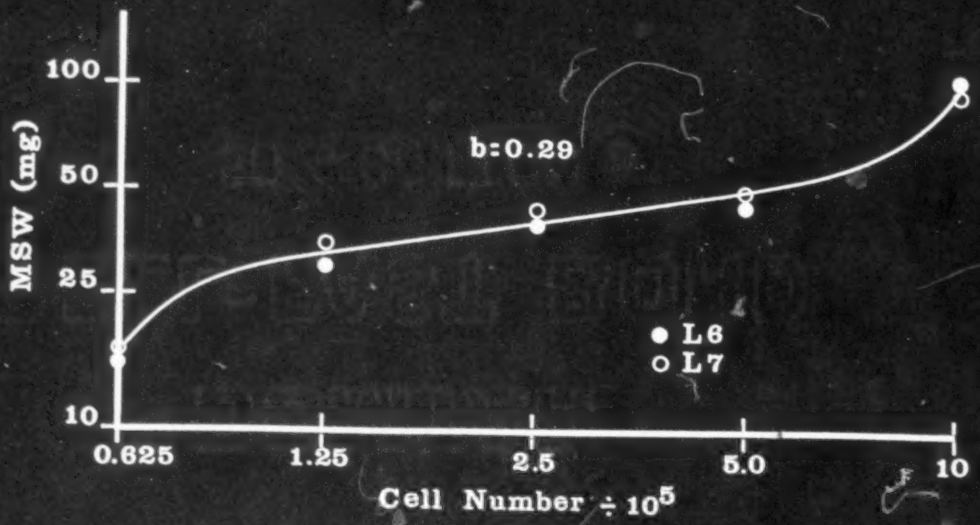


Figure 16. The limitation of GvH splenomegaly is directly related to the concentration of antiserum in which the PBLs are incubated. The upper line (b, A7xL7 = -0.779), two experiments that were pooled together, shows the decrease in mean embryo spleen weight (MSW) with an increase in the concentration of A7 in which L7 PBLs were incubated. The lower line (b, A6xL6 = -0.700), two experiments that were pooled together, shows the decrease in mean embryo spleen weight with an increase in the antiserum concentration of A6 in which L6 PBLs were incubated. The vertical bars indicate the standard errors for each mean. The upper two points at the left margin (0%) represent the spleen weights of embryos inoculated with L7 or L6 PBLs incubated with NS. The lower two points at the left margin (0%) represent the spleen weights of uninjected embryos. The means and standard errors were obtained after transformation to $\log_{10}$. The middle portion of each curve is linear ($p<0.001$) and the slope refers to this region.

Figure 16

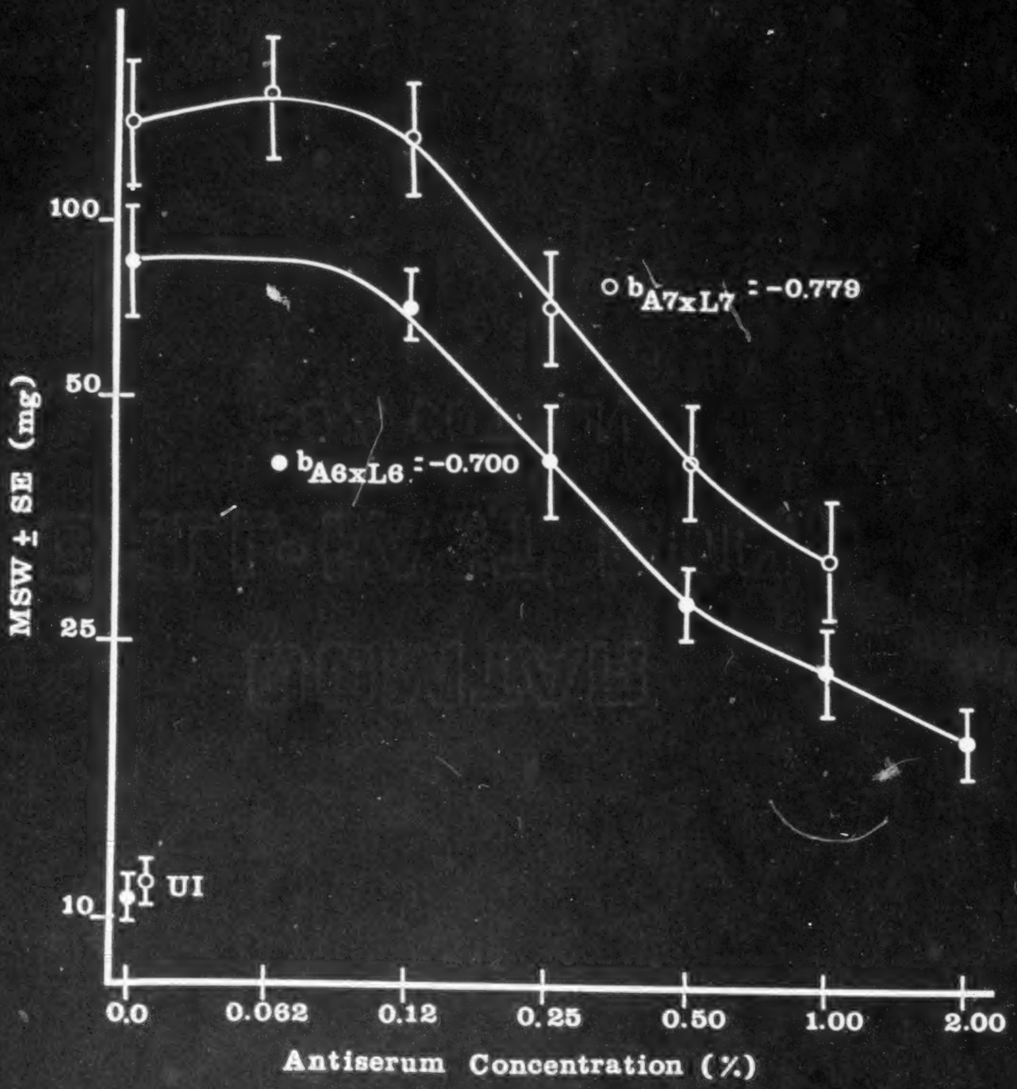

MSW ± SE (mg)
100
50
25
10
○ b_{A7xL7} = -0.779
● b_{A6xL6} = -0.700
UI
0.0
0.062
0.12
0.25
0.50
1.00
2.00
Antiserum Concentration (%)

Figure 17. The GvH reactivity of L6 PBLs after pretreatment *in vitro* with A6 serum absorbed with $2x10^8$ L6 PBLs/ml, L6 RBCs/ml, L7 PBLs/ml, or L7 RBC/ml. The last group (UI) represents the spleen weight of uninjected outbred embryos. The upper solid and dashed horizontal line represents the mean response^{+}SE of L6 PBLs pretreated *in vitro* with normal serum. The solid square indicates a significant increase ($p=0.05$) in the MSW of those embryos that were inoculated with L6 PBLs after pretreatment with the absorbed antiserum compared with the MSW of those chick embryos inoculated with L6 PBLs incubated with antiserum absorbed with L6 RBCs.

Figure 17

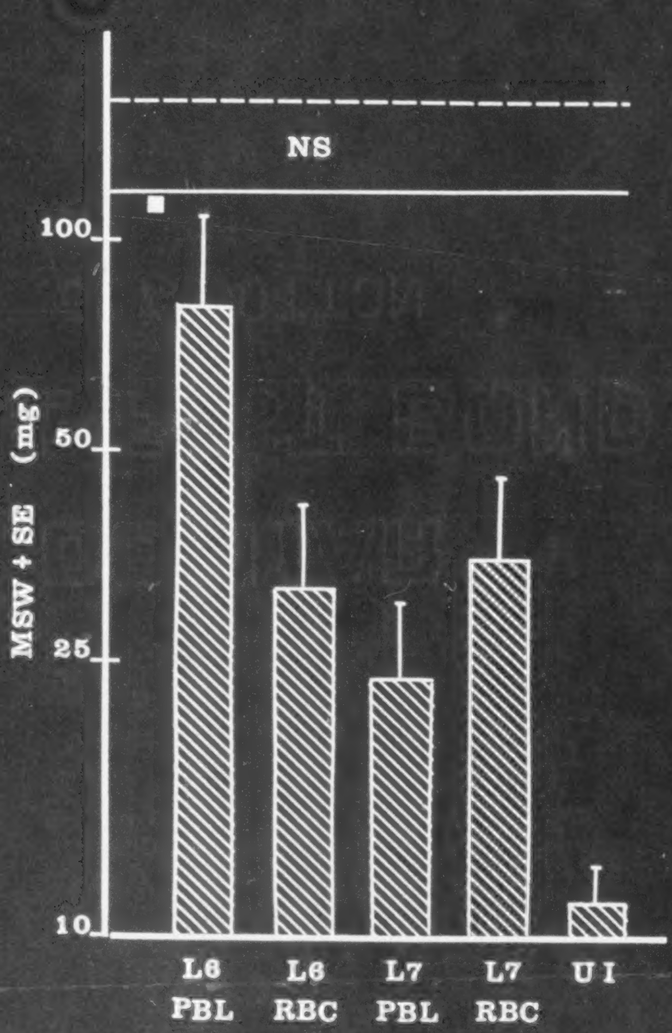
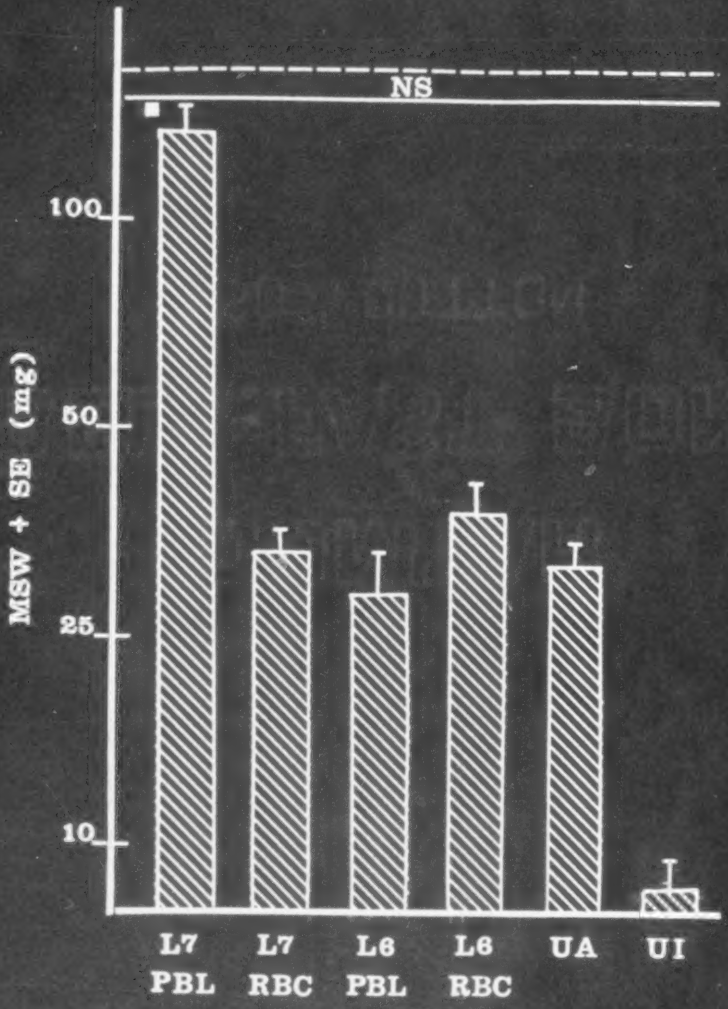


Figure 18. The GvH reactivity of L7 PBLs after pretreatment *in vitro* with A7 serum absorbed with 2x10^8 L7 PBLs/ml, L7 RBCs/ml, L6 PBLs/ml, or L6 RBCs/ml. The last group (UI) represents the MSW of uninjected embryos. The upper solid and dashed horizontal line represents the mean response of L7 PBLs pretreated *in vitro* with NS. The greater variation represented by the standard errors in Figure 17 as compared to Figure 18 occurs when random bred embryos are used for inoculation. The solid square indicates a significant increase ($p=0.05$) in the MSW of those embryos that were inoculated with L7 PBLs pretreated with the absorbed antiserum compared with the MSW of those chick embryos inoculated with L7 PBLs incubated with unabsorbed A7 serum.

Figure 18



Figures 19. The graph on the left (Figure 19a) represents the GvH reactivity of L7 PBLs after *in vitro* pretreatment with A7 serum absorbed with 2x10^{8} L7 PBLs/ml, Th cells/ml, Bu cell/ml, or RBCs/ml. The graph on the right (Figure 19b) represents the GvH reactivity of L7 PBLs after *in vitro* pretreatment with A7 serum absorbed with 4x10^{8} L7 spleen (Spl), Th, Bu, or RBCs. The upper solid and dashed horizontal lines represent the MSW+SE of embryos inoculated with L7 PBLs pretreated *in vitro* with normal serum. The solid square indicates a significant increase (p=0.05) in the MSW of those embryos inoculated with L7 PBLs after pretreatment with absorbed antiserum compared with the MSW of those embryos inoculated with L7 PBLs after pretreatment with unabsorbed antiserum.

Figure 19

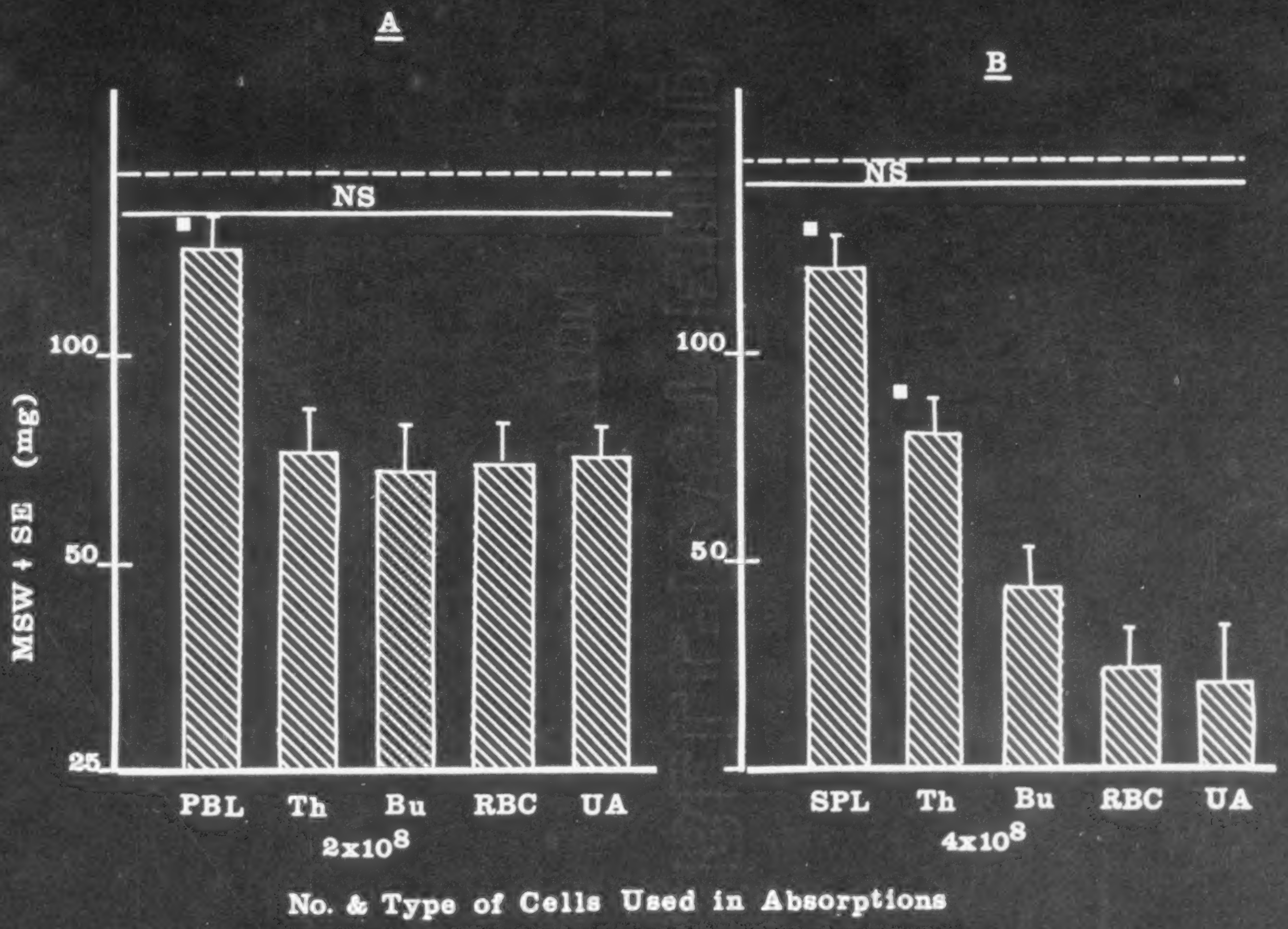

A
B
MSW ± SE (mg)
25
50
100
NS
PBL
Th
Bu
RBC
UA
2x10^8
SPL
Th
Bu
RBC
UA
4x10^8
No. & Type of Cells Used in Absorptions

Figure 20. The correlation between limiting and nonlimiting GvH splenomegaly of L6, L7, and F1 PBLs after incubation with NS, A6, or A7 sera. The upper line represents the principal axis of correlation of the mean log$_{10}$ mg embryo spleen weight (ordinate) evoked by PBLs treated with nonlimiting antiserum (A6xL7 and A7xL6) with the mean log$_{10}$ mg embryo spleen weight evoked by PBLs treated with NS (abscissa). The lower line represents the principal axis of correlation of the mean log$_{10}$ embryo spleen weight (ordinate) evoked by PBLs treated with limiting antisera (A6xL6, A6xF1, A7xF1, A7xL7). The mean difference between these lines is 0.476 which transformed from log$_{10}$ to the arithmetic, means that the limited GvH spleens are one-third the size of the non-limited GvH spleens. The upper line is based on 17 means (Table IX) and the lower line is based on 17 means for L6 and L7 and on 4 means for F1's. The slopes of these lines do not differ significantly from a slope of 1.0. None of the means for the limited GvH reactions overlap with those for the nonlimited GvH reactions at the same time.

Figure 20

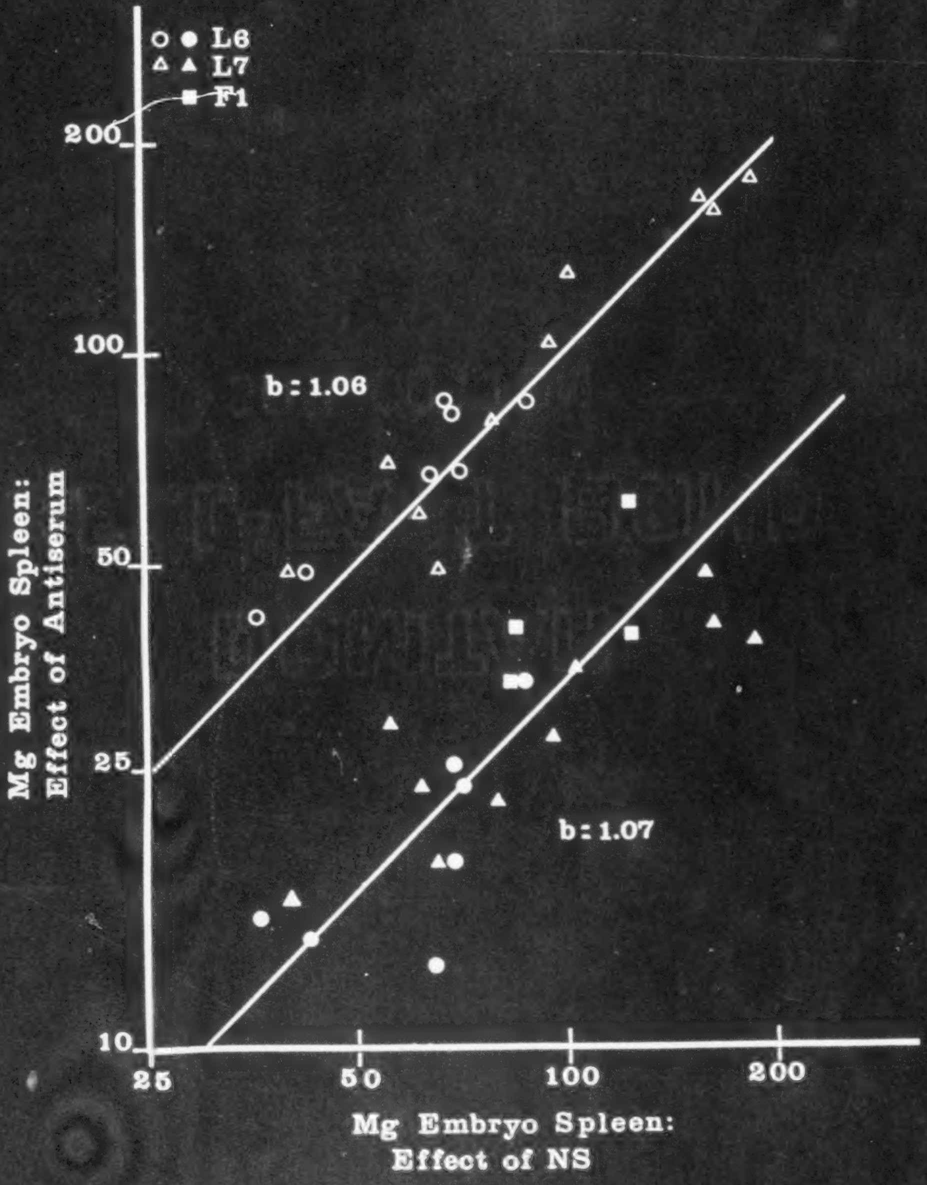

○ ● L6
△ ▲ L7
■ F1
200
100
50
25
10
25
50
100
200
b : 1.06
b : 1.07
Mg Embryo Spleen: Effect of Antiserum
Mg Embryo Spleen: Effect of NS

Figure 21. A frequency histogram illustrating the distribution of F2 chickens typed with A6 and A7 antisera. The F2 chickens were categorized using the differential limitation of GvH spleen weight (difference) represented on the abscissa. The number of F2 chickens that fit into each 0.1 units of the difference is represented on the ordinate. The observed mean for each class is signified by the black arrows. The vertical lines indicate the location of the division points sorting this F2 population into three lymphocyte antigen types, from left to right (L7-type, F1-type, and the L6-type). The means of the difference (differential limitation) of the three classes differed significantly from one another ($F_{2,98} = 317$, $p<0.001$).

Figure 21

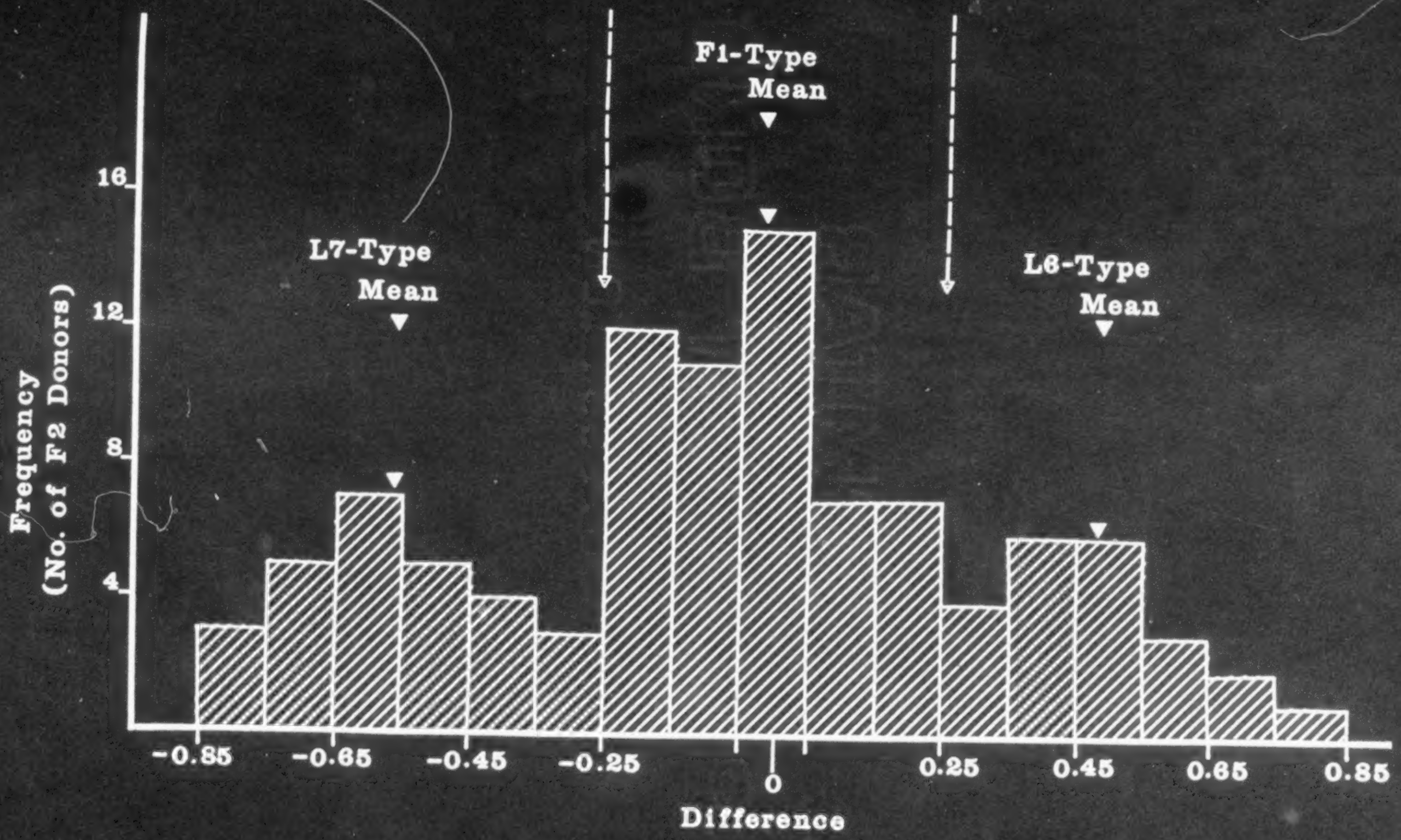

Frequency
(No. of F2 Donors)
4
8
12
16
L7-Type
Mean
▼
F1-Type
Mean
▼
L6-Type
Mean
▼
-0.85
-0.65
-0.45
-0.25
0
0.25
0.45
0.65
0.85
Difference

Figure 22. A probability graph of the distribution of F2, (L6xL7 parents), progeny typed with A6 and A7 sera. The differential limitation of the GvH spleen weight (ordinate, difference) for each F2 donor was plotted against the culmulative percent distribution of these donors (abscissa) ranking the lowest value of the difference as 1 and the highest as 99. The graph is designed for examination of 99 points therefore, the first 99 values of the difference from the 101 tested progeny are represented here. If the F2 population consist of only one class of individuals the graph will show a straight line as indicated for the data by the dashed line. If there are two classes the graph will show a sigmoidal line, and if three, a doubly inflected line. The graph of the F2 progeny shows a sigmoidal line. The mean difference for the F2 L7-type, (putative Ly-4.2/Ly-4.2), the F1-type (putative Ly-4.1/Ly-4.2), and the L6-type (putative Ly-4.1/Ly-4.1) are shown by the arrows and arrow tips to the right of the Mean. The second sigmoidal line (above and below those of the F2) represent the approximate distribution for the parental L6 and L7 donors. The arrows signify the location of the mean difference for these groups.

Figure 22

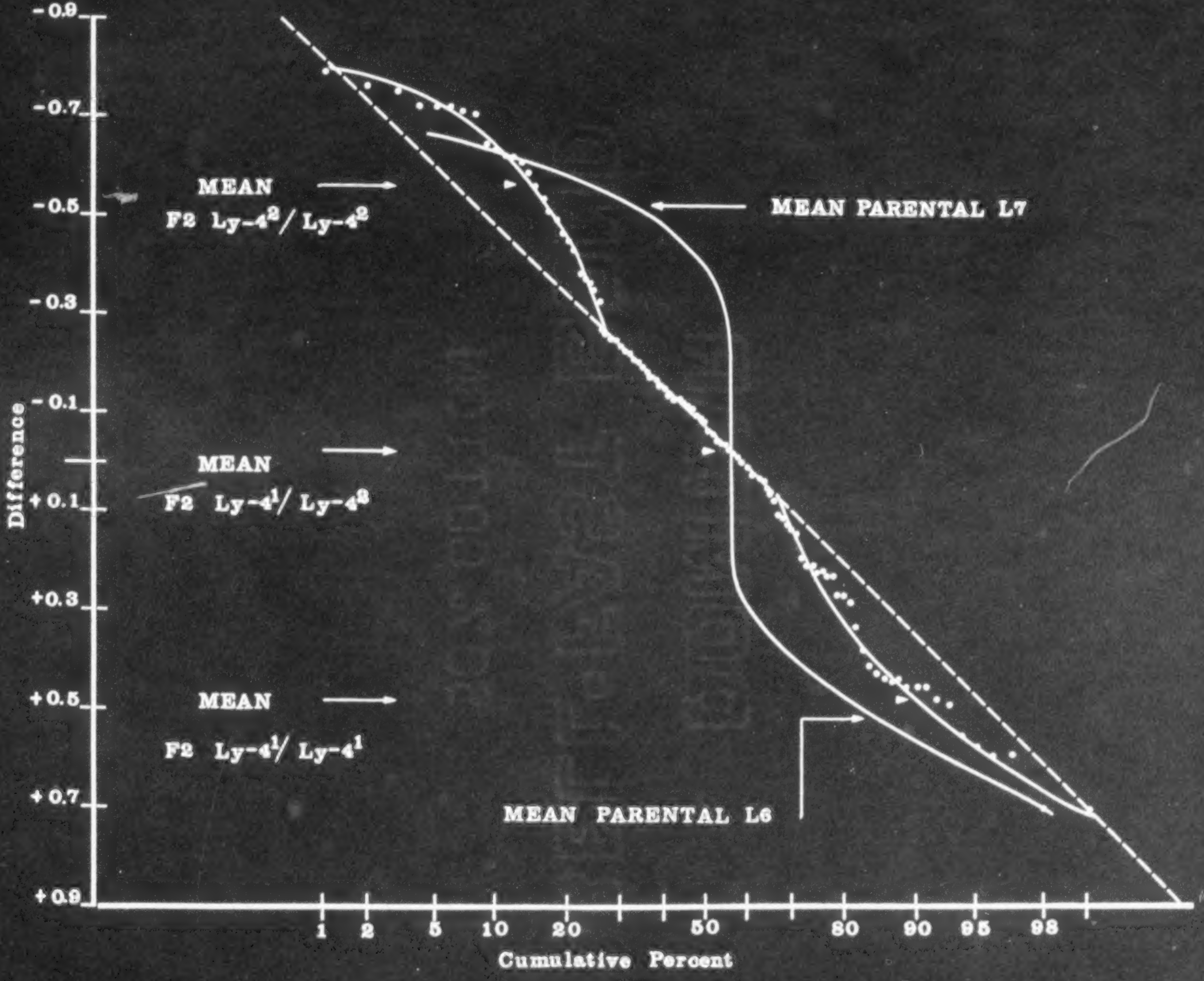
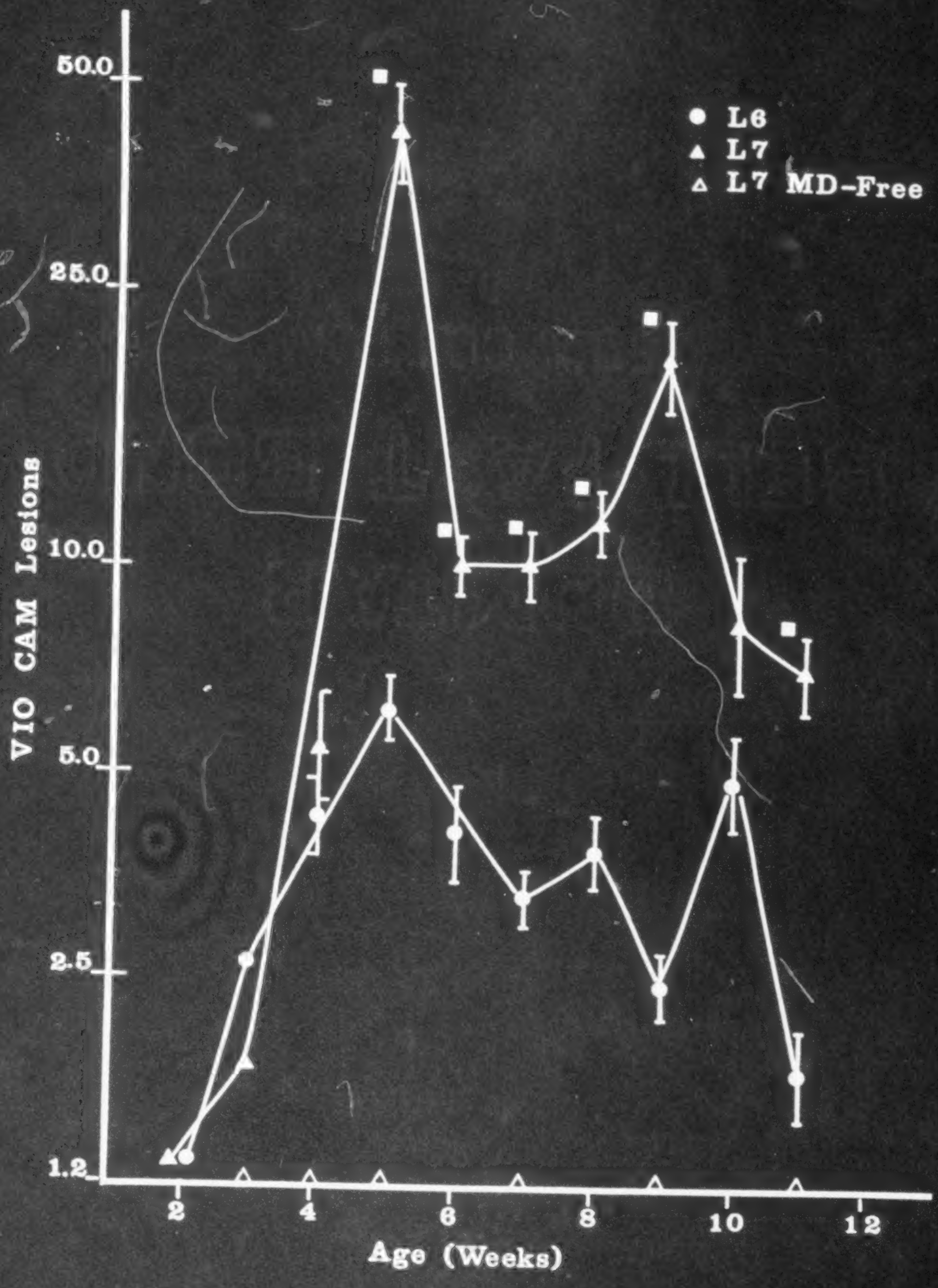

Difference
- 0.9
- 0.7
- 0.5
- 0.3
- 0.1
+ 0.1
+ 0.3
+ 0.5
+ 0.7
+ 0.9
MEAN
F2 Ly-4²/ Ly-4²
MEAN
F2 Ly-4¹/ Ly-4²
MEAN
F2 Ly-4¹/ Ly-4¹
MEAN PARENTAL L7
MEAN PARENTAL L6
1
2
5
10
20
50
80
90
95
98
Cumulative Percent

Figure 23. Assay of MDV in the blood of parental L6 and L7 donors infected with MDV at hatching. Each group of chickens were bled weekly and the blood (1:4 dilution) was inoculated into $\underline{B}^2/\underline{B}^2$ 12-day chick embryos. The mean number of MDV, CAM lesions per 0.1 of inoculated blood (abscissa) was plotted against the age of the donors. The solid square represents a significant difference ($p<0.01$) between the number of viral lesions formed on the CAM by L6 and L7 MDV blood cells.

Figure 23



50.0
25.0
10.0
5.0
2.5
1.2
VIO CAM Lesions
2
4
6
8
10
12
Age (Weeks)
● L6
▲ L7
△ L7 MD-Free

BIBLIOGRAPHY

Albini, B. and G. Wick. 1973. Immunoglobulin determinants on the surface of chicken lymphoid cells. Int. Arch. Allergy 44: 804-822.

Albini, B. and G. Wick. 1974. Delineation of B and T lymphoid cells in the chicken. J. Immunol. 112: 444-450.

Aoki, T., U. Hammerling, E. de Harven, E. A. Boyse, and L. J. Old. 1969. Antigenic structure of cell surfaces. An immunoferritin study of the occurrence and topography of H-2, θ, and TL alloantigens on mouse cells. J. Exp. Med. 130: 979-1001.

Aoki, T., I. F. C. McKenzie, M. M. Sturm, and M. Liu. 1974. Distribution of the alloantigen Ly-4.2 on murine B cells. Immunogenetics 3: 291-296.

Aspinall, R. L. and R. K. Meyer. 1964. Effect of steroidal and surgical bursectomy and surgical thymectomy on the skin homograft reaction in chickens. In: The Thymus In Immunobiology, R. A. Good and A. E. Gabrielsen, eds. Harper and Row, New York. Pp. 376-392.

Bankhurst, A. D., B. T. Rouse, and N. L. Warner. 1972. Surface immunoglobulins on bursa-derived chicken lymphoid cells. Int. Arch. Allergy 42: 187-195.

Boak, J. L., M. Fox, and R. E. Willson. 1967. Activity of lymphoid tissues from antilymphocyte-serum treated mice. Lancet I: 750-752.

Boyse, E. A. and L. J. Old. 1969. Some aspects of normal and abnormal cell surface genetics. Ann. Rev. Genet. 3: 269-290.

Boyse, E. A., K. Itakura, E. Stockert, C. A. Iritana, and M. Miura. 1971. A third locus specifying alloantigens expressed only on thymocytes and lymphocytes. Transplantation 11: 351-353.

Boyse, E. A., M. Miyazawa, T. Aoki, and L. J. Old. 1968. Ly-A and Ly-B. Two systems of lymphocyte isoantigens in the mouse. Proc. roy. Soc. B. 170: 175-193.

Boyse, E. A., L. J. Old, and E. Stockert. 1965. The TL (thymus leukemia) antigen. A review. In: IV Int. Symp. Immunopathology, P. Grabar and P. A. Miescher, eds. Grune and Stratton, Inc, New York. Pp. 23-40.

Bøyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21 (Suppl. 97): 77-89.

Brent, L., T. Courtenay, and G. Gowland. 1967. Immunological reactivity of lymphoid cells after treatment with anti- lymphocytic serum. Nature 215: 1461-1464.

Brewer, R. N., C. H. Moore, and L. W. Johnson. 1969 (abstract). Effects of blood group antigens on resistance to Marek's disease. Poultry Sci. 48: 1790.

Briles, W. E. and W. L. Oleson. 1971 (abstract). Differential depletion of B blood group genotypes understress of Marek's disease. Poultry Sci. 50: 1558.

Cantor, H. and E. A. Boyse. 1975a. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. J. Exp. Med. 141: 1376-1389.

Cantor, H. and E. A. Boyse. 1975b. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclassis of Ly^+ cells in the generation of killer activity. J. Exp. Med. 141: 1390-1399.

Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Thymus-marrow cell combinations. Synergism in antibody production. Proc. Soc. Exp. Biol. Med. 122: 1167-1171.

Coppleson, L.W. and P. Michie. 1966. A quantitative study of the chorioallantoic membrane reaction in the chick embryo. Proc. Roy. Soc. B 163: 555-563.

Cooper, M. D., R. D. A. Peterson, M. A. South, and R. A. Good. 1966. The functions of the thymus and bursa system in the chicken. J. Exp. Med. 123: 75-102.

Crittenden, L. B., R. L. Muhn, and B. R. Burmester. 1972. Genetic control of susceptibility to the avain leukosis complex. 2. Marek's disease. Poultry Sci. 51: 261-267.

Crone, M., C. Kock, and M. Simonsen. 1972. The elusive T cell receptor. Transplant. Rev. 10: 36-56.

Dalmasso, A. P., C. Martinez, and R. A. Good. 1964. Studies of immunologic characteristics of lymphoid cells from thymectomized mice. In: The Thymus In Immunobiology, R. A. Good and A. E. Gabrielsen, eds. Harper and Row, New York. Pp. 478-488.

Forget, A., E. F. Potworowski, G. Richer, and A. G. Borduas. 1970. Antigenic specificities of bursal and thymic lymphocytes in the chicken. Immunology 19: 465-468.

Gallagher, M. T., E. R. Richie, L. R. Heim, K. Judd, and J. J. Trentin. 1972. Inhibition of the graft-vs-host reaction. I. Reduction of the graft-versus-host potential of mouse spleen cells (with a sparing of stem cells) by treatment with antilymphocyte globulin-derived Fab fragments. Tranplantation 14: 597-602.

Gigli, I. and K. F. Austin. 1971. Phylogeny and function of the complement system. Ann. Rev. Microbiol. 25: 309-332.

Gilmour, D. G., A. Brand, N. Donnelly, and H. A. Stone. 1976. Bu-1 and Th-1, two loci determining surface antigens of B or T lymphocytes in the chicken. Immunogenetics, in press.

Glick, B., T. S. Chang, and R. G. Jaap. 1956. The bursa of Fabricius and antibody production. Poultry Sci. 35: 224-226.

Greaves, M. F., A. Tursi, J. H. L. Playfair, G. Torrigiani, R. Zamir, and I. M. Roitt. 1969. Immunosuppressive potency and in-vitro activity of antilymphocyte globulin. Lancet 1: 68-72.

Hansen, M. P., J. N. Van Zandt, and G. R. J. Law. 1967 (abstract). Differences in susceptibility to Marek's disease in chickens carrying two different B locus blood group alleles. Poultry Sci. 46: 1268.

Hudson, L. and I. M. Roitt. 1973. Immunofluorescent detection of surface antigens specific to T and B lymphocytes in the chicken. Eur. J. Immunol. 3: 63-67.

Itakura, K., J. Hutton, E. A. Boyse, and L. J. Old. 1971. Linkage groups of the 0 and Ly-A loci. Nature (New Biology) 230: 126.

Itakura, K., J. J. Hutton, E. A. Boyse, and L. J. Old. 1972. Genetic linkage relationships of loci specifying differentiation alloantigens in the mouse. Transplantation 13: 239-243.

Ivanyi, J. and P. M. Lydyard. 1972. Delineation of chicken lymphocyte populations by specific anti-thymus and anti-bursa sera. Cellular Immunol. 5: 180-189.

Jankovic, B. D., K. Isakovic, S. Petrovic, and D. Vujic. 1970a. Studies on antilymphocyte antibody in the chicken. I. Effect of rabbit antithymus and antibursa globulin on chicken embryos. Clin. exp. Immunol. 7: 693-707.

Jankovic, B. D., K. Isakovic, S. Petrovic, D. Vujic, and J. Horvat. 1970b. Studies on antilymphocyte antibody in the chicken. II. Effect of rabbit antithymus and antibursa globulin on immune reactions in young chickens. Clin. exp. Immunol. 7: 709-722.

Kincade, P. N., A. R. Lawton, and M. D. Cooper. 1971. Restriction of surface immunoglobulin determinants to lymphocytes of the plasma cell line. J. Immunol. 106: 1421-1423.

Klein, J. 1975. Biology of the Mouse Histocompatibility -2 Complex. Springer-Verlag, New York.

Komuro, K., K. Itakura, E. A. Boyse, and M. John. 1975. Ly-5: A new T lymphocyte antigen system. Immunogenetics 1: 452-456.

Lance, E. M., P. B. Medawar, and R. N. Taub. 1973. Antilymphocyte serum. In: Advances in Immunology, F. J. Dixon and H. G. Kunkel, eds. Academic Press, New York. Vol. 17, pp. 1-92.

Levey, R. H. and P. B. Medawar. 1967. The mode of action of antilymphocytic serum. In: Antilymphocyte Serum, G. E. W. Wolstenholme and M. O'Connor, eds. Little, Brown, and Co., Boston. Pp. 72-84.

Longenecker, B. M., F. Pazderka, J. S. Gavora, J. L. Spencer, and R. F. Ruth. 1976. Lymphoma induced by herpesvirus: Resistance associated with a major histocompatibility gene. Immunogenetics 3: 401-407.

Longenecker, B. M., F. Pazderka, G. R. J. Law, and R. F. Ruth. 1972. Genetic control of graft-versus-host competence. Transplantation 14: 424-431.

Longenecker, B. M., F. Pazderka, H. S. Stone, J. S. Gavora, and R. F. Ruth. 1975. _In ovo_ assay for Marek's disease virus and turkey herpesvirus. Infect. Immun. _11_: 922-931.

Longenecker, B. M., S. Sheridan, G. R. J. Law, and R. F. Ruth. 1970. Measurement of the graft-vs-host reactions on the chorioallantoic membrane of the chicken. Transplantation _9_: 544-557.

Lydyard, P. and J. Ivanyi. 1971. Suppression of graft-versus-host-reactive lymphocytes by heterologous anti-thymus serum _in vitro_. Transplantation _12_: 493-499.

Lydyard, P. M. and J. Ivanyi. 1974. The role of opsonization in antithymocyte globulin-induced suppression of graft-versus-host reactions in chick embryos. Transplantation _17_: 400-404.

Lydyard, P. M. and J. Ivanyi. 1975. Chimaerism of immuno-competent cells in allogeneic bone marrow-reconstituted lethally irradiated chickens. Transplantation _20_: 155-162.

Martin, W. J. 1969. Assay for the immunosuppressive capacity of antilymphocyte serum. I. Evidence for opsonization. J. Immunol. _103_: 979-989.

Martin, W. J. and J. F. A. P. Miller. 1967. Site of action of antilymphocyte globulin. Lancet _2_: 1285-1287.

McArthur, W. P., J. Chapman, and G. J. Thorbecke. 1971. Immunocompetent cells in the chicken. I. Specific surface antigenic markers on bursa and thymus cells. J. Exp. Med. _134_: 1036-1045.

McBride, R. A., L. W. Coppleson, N. W. Nisbet, M. Simonsen, A. Skowron-Cendzak, and H. L. R. Wigzell. 1966. Accelerated immunological maturation in the chick. Immunology _10_: 63-79.

McKenzie, I. F. C. 1975. Ly-4.2: A cell membrane allo-antigen of murine B lymphocytes. II. Functional studies. J. Immunol. _114_: 856-862.

McKenzie, I. F. C. and G. D. Snell. 1975. Ly-4.2: A cell membrane alloantigen of murine B lymphocytes. I. Population studies. J. Immunol. _114_: 848-855.

Miller, J. F. A. P. 1964. Effect of thymic ablation and replacement. In: _The Thymus In Immunobiology_, R. A. Good and A. E. Gabrielsen, eds. Harper and Row, New York. Pp. 436-460.

Mitchell, G. F. and J. F. A. P. Miller. 1968. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. J. Exp. Med. 128: 821-837.

Mueller, A. P., H. R. Wolfe, and W. P. Cote. 1964. Antibody studies in hormonally and surgically bursectomized chickens. In: The Thymus In Immunobiology, R. A Good and A. E. Gabrielsen, eds. Harper and Row, New York. Pp. 359-373.

Nossal, G. J. V., A. Cunningham, G. F. Mitchell, and J. F. A. P. Miller. 1968. Cell to cell interaction in the immune response. III. Chromosomal marker analysis of single antibody-forming cell in reconstituted, irradiated, or thymectomized mice. J. Exp. Med. 128: 839-853.

Old, L. J., E. A. Boyse, and E. Stockert. 1963. Antigenic properties of experimental leukemias. I. Serological studies in vitro with spontaneous and radiation induced leukemias. J. Nat. Cancer Inst. 31: 977-986.

Pazderka, F., B. M. Longenecker, G. R. J. Law, and R. F. Ruth. 1975a. Review: The major histocompatibility complex of the chicken. Immunogenetics 2: 101-130.

Pazderka, F., B. M. Longenecker, G. R. J. Law, H. A. Stone, and R. F. Ruth. 1975b. Histocompatibility of chicken populations selected for resistance to Marek's disease. Immunogenetics 2: 93-100.

Potworowski, E. F. 1972. T and B lymphocytes. Organ and age distribution in the chicken. Immunology 23: 199-204.

Potworoski, E. F., G. Richer, A. Borduas, and A. Forget. 1971. Selective inhibition of plaque forming cells by bursa specific antisera. J. Immunol. 106: 1416-1417.

Potworowski, E. F., J. D. Zavallone, J. C. Gilker, and G. Lamoureux. 1971. Inhibition of the graft-versus-host reaction by thymus-specific antibodies. Rev. Europ. E'tudes Clin. ET Biol. XVI: 155-157.

Rabellino, E. F. and H. M. Grey. 1971. Immunoglobulins on the surface of lymphocytes. III. Bursal origin of surface immunoglobulins on chicken lymphocytes. J. Immunol. 106: 1418-1420.

Raff, M. C. 1969. Theta isoantigen as a marker of thymus-derived lymphocytes in mice. Nature 224: 378.

Raff, M. C. 1971. Surface antigenic markers for distinguishing T and B lymphocytes in mice. Transplant. Rev. 6: 52-80.

Raff, M. C., S. Nase, and N. A. Mitchison. 1971. Mouse-specific B lymphocyte antigen (MSLA): A marker for thymus-independent lymphocytes. Nature 230: 50-51.

Raff, M. C., M. Sternberg, and R. B. Taylor. 1970. Immunoglobulin determinants on the surface of mouse lymphoid cells. Nature 225: 553-554.

Reif, A. E. and J. M. V. Allen. 1963. Specificity of isoantisera against leukemic and thymic lymphocytes. Nature 200: 1332.

Reif, A. E. and J. M. V. Allen. 1964. The AKR thymic antigen and its distribution in leukemias and nervous tissue. J. Exp. Med. 120: 413-433.

Rice, C. E. 1947. Atypical behavior of certain avian antisera in complement fixation tests. Can. J. Comp. Med. 11: 236-245.

Rice, C. E. and C. N. Crowson. 1950. The interchangeability of the complement components of different animal species. II. In the hemolysis of sheep erythrocytes sensitized with rabbit amboreceptor. J. Immunol. 65: 201-210.

Richie, E. R., M. T. Gallagher, J. J. Trentin. 1973. Inhibition of the graft-versus-host reaction. II. Prevention of acute graft-versus-host mortality by Fab fragments of antilymphocyte globulin. Transplantation 15: 486-491.

Richie, E. R., H. J. Monie', J. J. Trentin, and R. N Taub. 1975. Inhibition of the graft-versus-host reaction. III. Altered in vivo distribution of spleen cells pretreated with Fab fragments of antithymocyte globulin. Transplantation 19: 115-120.

Rose, M. E. and E. Orlans. 1962. Fowl antibody: III. Its haemolytic activity with complement of various species and some properties of fowl complement. Immunology 5: 633-641.

Rouse, B. T. and N. L. Warner. 1972. Suppression of graft versus host reactions in chickens by pretreatment of leucocytes with anti-light chain sera. Cellular Immunol. 3: 470-477.

Ruth, R. F., E. O. Hohn, and G. R. J. Law. 1975. Ontogeny of the lymphoid tissue. Scand. J. Haematol., Series Haematologica 8: 29-40.

Schierman, L. W. and A. W. Nordskog. 1961. Relationship of blood type to histocompatibility in chickens. Science 134: 1008-1009.

Schierman, L. W. and A. W. Nordskog. 1962. Relationship of erythrocyte to leucocyte antigen chickens. Science 137: 620.

Schierman, L. W. and A. W. Nordskog. 1965. Evidence for a second blood group histocompatibility system in chickens. Transplantation 3: 44-48.

Schlesinger, M. 1972. Antigens of the thymus. In: Progress in Allergy, P.Kallos, B. Waksman, and A. de Weck, eds. S. Karger, Basel. Vol. 16, pp. 214-299.

Schlesinger, M. and I. Yron. 1970. I. Serological demonstration of a thymus-dependent population of lymph node cells. J. Immunol. 104: 798-804.

Seto, F. and J. F. Albright. 1965. An analysis of host and donor contributions to splenic enlargement in chick embryos inoculated with adult chicken spleen cells. Develop. Biol. 11: 1-24.

Shigeno, N., C. Arpels, U. Hammerling, E. A. Boyse, and L. J. Old. 1968. Preparation of lymphocyte specific antibody from anti-lymphocyte serum. Lance II: 320.

Simonsen, M. 1962. Graft versus host reaction. Their natural history, and applicability as tools of reserach. In: Progress in Allergy, P. Kallos and B. H. Waksman, eds. S. Karger, Basel. Vol. 6, pp. 349-467.

Simonsen, M. 1973. Tissue typing: An experimental solution in the chicken. J. Immunol. 111: 1607-1609.

Simonsen, M. 1975. Allo-aggression in chickens. Acta. path. microbiol. scand. Sect. C, 83: 1-14.

Snell, G. D., M. Cherry, I. F. C. McKenzie, and D. W. Bailey. 1973. Ly 4, a new locus determining a lymphocyte cell surface alloantigen in mice. Proc. Natl. Acad. Sci. 70: 1108-1111.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco.

Steel, R. G. D. and J. H. Torrie. 1960. Principals and Procedures of Statistics. McGraw-Hill Co., New York.

Stone, H. A. 1975. Use of highly inbred chickens in research. U.S. Dept. Agric., A. R. S. Tech. Bull No. 1514.

Takahashi, T., L. J. Old, and E. A. Boyse. 1970. Surface alloantigens of plasma cells. J. Exp. Med. 131: 1325-1341.

Takahashi, T., L. J. Old, K. R. McIntire, and E. A. Boyse. 1971. Immunoglobulin and other surface antigens of cells of the immune system. J. Exp. Med. 134: 815-832.

Taub, R. N. and E. M. Lance. 1968. Effects of heterologous anti-lymphocyte serum on the distribution of ^{51}Cr-labeled lymph node cells in mice. Immunology 15: 633-642.

Terasaki, P. I. 1959. Identification of the type of blood-cell responsible for the graft-vs-host reactions in chicks. J. Embryol. Exp. Morph. 7: 394-408.

Toben, H. R. and R. L. St. Pierre. 1972. Alteration of immunological reactivity in chickens by the in ovo administration of antisera against central lymphoid tissue. Int. Arch Allergy 42: 454-465.

Tucker, D. F. 1968. Effects of anti-spleen serum on skin allograft survival and blood lymphocyte reactivity in the chicken. Nature 218: 1259-1261.

Warner, N. L. and A. Szenberg. 1964. Immunologic studies on hormonally bursectomized and surgically thymectomized chickens: Dissociation of immunologic responsiveness. In: The Thymus In Immunobiology, R. A. Good and A. E. Gabrielsen, eds. Harper and Row, New York. Pp. 395-411.

Waters, N. F. 1954. Avian lymphomatosis mortality among inbred line crosses. Tenth World's Poult. Cong. P. 201.

Weber, W. T. 1975. Avian B lymphocyte subpopulations: Origins and functional capabilities. Transplant. Rev. 24: 113-158.

Wick, G., B. Albini, and F. Milgrom. 1973. Antigenic surface determinants of chicken lymphoid cells. I. Serological properties of anti-bursa and anti-thymus sera. Clin. exp. Immunol. 15: 237-249.

Wick, G., B. Albini, and W. Johnson. 1975. Antigenic surface determinants of chicken lymphoid cells. II. Selective in vivo and in vitro activity of anti-bursa and anti-thymus sera. Immunology 28: 305-313.

Wick, G., J. H. Kite, and R. C. Cole. 1971. Suppression of spontaneous autoimmune thyroiditis in Obese strain (OS) chickens with anti-bursa and anti-thymus sera. Int. Arch. Allergy 40: 603-612.

Wong, S. Y., F. Pazderka, B. M. Longenecker, G. R. J. Law, and R. F. Ruth. 1972. Immobilization of lymphocytes at surfaces by alloantibodies. Immunol. Commun. 1: 597-613.

Wong, S. Y. 1974. Adherence of chicken lymphocytes to surfaces. PhD. Thesis. University of Alberta.